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Thoracic masses

Stigt, Johannes Adrianus

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Thoracic masses
From chest radiography and ultrasound guided biopsies to molecular biology

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Promotor:	Prof.dr. H.J.M.Groen
Beoordelingscommissie:	Prof.dr. H.C. Hoogsteden Prof.dr. H.A.M. Kerstjens Prof.dr. W. Timens

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From chest radiography and ultrasound guided biopsies to molecular biology

Jos Stigt

Paranimfen:

G.J. Bosman
J.L. Stigt

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Mieke de Haan

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Chapter 1

Introduction

Recent history of pulmonary oncologic diagnostics in general and in the Isala Clinics

INTRODUCTION

Recent history of pulmonary oncologic diagnostics in general and in the Isala Clinics

The analysis of patients with suspected thoracic malignancies is a daily activity for pulmonologists. Over the last decades the diagnostic arsenal of our profession broadened extensively and resulted in improved patient care. In this introduction a few landmark developments will be addressed because of their huge influence on thoracic oncology. The excitement about new diagnostic equipment and their strategic patient-orientated application encouraged research in this field and finally writing this thesis.

The first landmark development is the introduction of positron-emission tomography (PET) in the mid-nineties. Before that time, starting in the early seventies, imaging of presumed thoracic malignancies leaned mainly on computed tomography (CT). With the improvements of helical scanning in 1991 and subsequent increasing numbers of detectors, smaller lesion became visible and the anatomic delineation for tumors was sharper as ever before [1]. Although superior in anatomical detailing, metabolic information was lacking.
PET with radiolabeled fluorodeoxyglucose (FDG) provided this functional quality and was incorporated so gradually took its position in the diagnostic approach of lung cancer next to CT. The high impact of FDG-PET on detecting thoracic malignancies was described in several studies [2-4]. FDG-PET enabled the differentiation of benign from malignant lesions, the detection of (occult) distant metastatic disease, provided a more accurate mediastinal staging, disease monitoring and determination of recurrence than before.
It took several years before PET and CT were integrated into one instrument. Integration of both modalities resulted in superior tumor and nodal staging[5].
In 2005 an integrated PET-CT was installed in the Isala Clinics improving the accessibility and applicability enormously.

The second landmark development was the endoscopic technique that arose originally in the field of gastroenterology in the late nineties. Endoscopic ultrasound guided fine-needle aspirations (EUS-FNA) appeared to be extremely useful for the analysis of leftsided mediastinal and subcarinal abnormalities and upper abdominal masses. Therefore, EUS found its way to disciplines such as pulmonology and especially thoracic oncology [6, 7].
The diagnostic performance of EUS-FNA was studied extensively in subsequent years and impressive sensitivities and specificities were reported [8].
In 2005 EUS became available in Isala Clinics and was implemented by skilled pulmonologists. Isala Clinics became a regional center for EUS and many patients from referring hospitals were analyzed.

The role of PET-CT and EUS-FNA in relapsing lung cancer patients has been studied. There was, and there still is, much debate about the feasibility and relevance of restaging lung cancers after induction treatment. Safer surgery with support of an excellent intensive care unit made it possible to perform surgery in more extensive stages but were we able to stage those patients reliably enough to decide whether any treatment would be beneficial? The almost simultaneous appearance of FDG-PET and EUS made it possible to focus on the value of restaging in lung cancer after concurrent chemoradiotherapy. These results are described in chapter 2.

EUS-FNA is a very safe procedure also as a restaging procedure. Complications are rare and usually mild. Is EUS-FNA also safe in patients treated with an angiogenesis inhibitor such as bevacizumab? Bevacizumab is used in combination with chemotherapy as first-line treatment in NSCLC. It is known for its side-effect of impaired wound-healing. Chapter 4 describes a patient who underwent EUS-FNA while on treatment with bevacizumab.

EUS is not only suitable for diagnosing malignant disease but has also a role in benign diseases such as sarcoidosis [9]. Other benign diseases can be diagnosed with EUS and are reported in literature [10]. As an example we report on two patients with infectious spondylodiscitis diagnosed with EUS and who are described in chapter 3. The cases illustrate the accessibility of posterior located mediastinal structures – previously inaccessible except by major surgery - and these cases emphasize the importance of cross-over knowledge between different medical disciplines.

While getting familiar with transesophageal and transgastric ultrasound (US) images, the step to percutaneous US guided sampling was rapidly made. In 2006 the first percutaneous ultrasound guided aspirations by pulmonologists in Isala Clinics were performed in supraclavicular lymph nodes. Many patients followed and percutaneous ultrasonography is now fully integrated in our diagnostic process.
The diagnostic performance characteristics of percutaneous US guided biopsies and aspirations of abnormalities detected with PET-CT are described in chapter 5. The role of percutaneous US guided sampling in staging is illustrated as well as its usefulness in non-palpable lesions.

To extent the use of ultrasound guided biopsies we looked to patients with suspected mesothelioma and in whom it was difficult to get a histological diagnosis. In this subgroup of mesothelioma patients, characterized by the absence of pleural effusion, it is hardly feasible to perform a medical thoracoscopy. Since tissue is required for diagnosing mesothelioma, a study of the diagnostic accuracy and safety of US guided TCB in this particular subgroup of patients was performed. The results are described in chapter 6.

A few years after EUS, the endobronchial variant made its appearance, partly overlapping and complementary to EUS in enabling the analysis of the right-sided

mediastinum and hilar lymph nodes [11, 12]. EBUS has a likewise excellent diagnostic performance in the hands of experienced operators [13].
Just like EUS, EBUS is a new and important tool for staging the mediastinum and the combined use of both techniques has driven surgical staging to the background [14].
In August 2008 endobronchial ultrasound (EBUS) was operational in Isala Clinics and patients from the wide region were referred.

A substantial amount of patients were referred for mediastinal lymphadenopathy detected incidentally on CT scans made for other purposes than cancer analysis such as pulmonary embolism or coronary arterial disease. With the increasing use of advanced CT technology for all kinds of indications and the developments in lung cancer screening ahead with low-dose CT, we expected increasing numbers of referrals for these so-called mediastinal incidentalomas. The question arose whether it is useful to further analyse these thoracic abnormalities? A prospective study was performed on these incidental findings by characterizing them radiologically and pathologically. Results are described in chapter 7.

This state-of-the-art diagnostics provided the opportunity for a fast-track diagnostic program for patients with an abnormal chest radiograph suspected for lung cancer. The core of this program is the immediate availability of invasive techniques after FDG-PET performed at the same day. However, is this fast diagnostic approach really of benefit for the patient and is it really faster? The results of this diagnostic program are described in chapter 8.

The third diagnostic landmark was the introduction of molecular diagnostics into pulmonary oncology. For a long time lung cancer was divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The diagnosis was based on morphology with haematoxylin and eosin stained slides from tumor tissue.
In 2008 a study demonstrated a relation between chemotherapeutical efficacy and tumor histology[15]. From that moment subtyping of NSCLC was essential to select the proper chemotherapy. Histology remained important but it was not always possible to obtain enough tumor tissue. Under those circumstances cytology was used to come to a diagnosis. Initially only cytologic aspirations were obtained for staging lung cancer but gradually, depending on the indication and the size of the lesion, also tissue core biopsies (TCB) were taken.
To use cytological specimen in more elaborate way, cytological aspirates had to be deposited in a fixative medium for the preparation of formalin fixed paraffin-embedded (FFPE) cell blocks. With these cell blocks and the use of immunocytochemical staining a diagnosis can be made. The question is however whether cytology or pathology specimen are required for an adequate lung cancer diagnosis.
In the late 2000s more and more evidence appeared that certain activating mutations in the epidermal growth factor receptor (EGFR) gene predicted good response to treatment with EGFR tyrosine kinase inhibitors (TKIs). DNA analysis would be essential in the near future and the first plans to start a molecular biology laboratory

in the Isala Clinics arose in 2007. After KRAS mutation analysis in colon carcinoma was made operational by the end of 2009, the first DNA isolation for lung cancers followed by the end of 2010.

The first study that showed a superior outcome of EGFR-TKIs over chemotherapy in first-line treatment of lung cancer patients with an activating EGFR mutation emphasized the importance of EGFR mutation analysis[16]. Many large subsequent studies showed comparable results. In these studies, mutation analysis was uniformly performed on tumor tissue with different molecular tests. The slogan “tissue is the issue” was widespread and a consensus report recommended mutation analysis to be performed preferably on tumor tissue[17].

As many of the diagnoses of our patients are based on cytologic material, a study was started to compare the usefulness of EUS guided aspirations with EUS guided TCBs for pathological classification and mutation analysis. The question was whether aspirations with adequate number of tumor cells are comparable with tumor tissue for molecular diagnosis. The result of such a comparison is described in chapter 9.

In recent years, lots of evidence appeared about the feasibility of mutation analysis on cytologic samples, on samples with less and less tumor cells. Even single cell genome-wide sequencing was introduced for research purposes. In our laboratory a very sensitive sequencing method was implemented that required very small tumor quantities. This so-called pyrosequencing method was tested systematically on EUS and EBUS aspirations and compared with the results of the High Resolution Melting (HRM) technique. The results are described in chapter 10.

The integration of all these diagnostic modalities into an appropriate diagnostic pathway for an individual patient is the art of the pulmonologist. A few principles should guide diagnostic decision-making to obtain maximal information from a minimal number of tests.
First, the clinical consequences and personal wishes of the patient should be considered before appointments are made.
Second, waiting in uncertainty about the diagnosis is tormenting for the patient, diagnosing time must be short.
Third, imaging should precede invasive tests. Imaging provides the location and extensiveness of disease and determines whether and how tumours should be sampled for pathological analysis depending on differential diagnostic considerations.
Fourth, invasive test should ideally provide a diagnosis and, in case of malignancy, a stage confirmation.
How can we diagnose patients suspected for thoracic malignancies without losing sight of these principles? In chapter 11, diagnostic tools and their practical implementation are described more in detail in order to highlight these issues.

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Chapter 2

Comparison of EUS-Guided Fine Needle Aspiration and integrated PET-CT in Restaging after Treatment for Locally Advanced Non-small Cell Lung Cancer

Lung Cancer 2009;66:198-204.

Jos A. Stigt, Ad H. Oostdijk, Paul R. Timmer, Ghada M. Shahin, James E. Boers, Harry J.M. Groen.

SUMMARY

Background*:* After induction treatment restaging of mediastinal disease in patients with stage III non-small cell lung cancer (NSCLC) may lead to selection of candidates for further surgical treatment. Nodal down-staging is the best predictive characteristic for proceeding with surgery.
We report our experience in restaging with endoscopic ultrasound-guided fine-needle aspirations (EUS-FNA) and with repeated integrated positron emission tomography and computed tomography (PET-CT).
Methods*:* Twenty-eight patients with stage III NSCLC were staged with integrated PET-CT, cerebral Magnetic Resonance Imaging (MRI) and pathologically proven nodal disease.
Restaging was performed with PET-CT and EUS-FNA on the same nodes that showed initially metastatic disease provided this nodal sites determined the tumor stage. Cerebral MRI was not repeated.
When restaging EUS-FNA revealed no malignant cells anymore, patients were operated. The postoperative pathologic results were compared with the pre-operative restaging EUS-FNA results. Also, patterns of decreased fluoro-2-deoxyglucose (FDG) uptake were compared with the postoperative pathologic results.
Results*:* Restaging EUS-FNA was well tolerated in all patients even in those with clinical signs of radiation esophagitis.
Of the 28 patients 15 were down-staged based on cytologic findings with restaging EUS-FNA and in one patient the cytology was not conclusive. Of these 15 patients, down-staging was histologically confirmed after mediastinal exploration in 11 patients and one patient had persistent nodal disease at resection. In 3 patients no mediastinal tissue verification was performed. Two subjects were not fit for operation, and in the other patient intraoperative nodal staging was omitted. The negative predictive value for restaging EUS-FNA was 91.6%. The accuracy of EUS-FNA was 92.3%.
Concordance between findings of restaging EUS-FNA and metabolic response of lymph node metastases occurred in 17 out of 27 patients.
Conclusion*:* Restaging with EUS-FNA after induction chemo(-radiotherapy) is well tolerated and predicts the absence of nodal metastasis reliably. Although changes in mediastinal FDG-PET uptake show a high concordance with EUS-FNA , pathological confirmation is still superior and therefore necessary. EUS-FNA is the procedure of first choice for mediastinal restaging.

INTRODUCTION

Several studies demonstrated that down-staging mediastinal node metastases predicted prolonged survival after induction therapy with chemotherapy and chemo-radiotherapy in patients with locally advanced Non-small Cell Lung Cancer (NSCLC)[1-6]. Concurrent chemoradiotherapy seems the most effective way to clear tumor cells from nodal stations in stage III disease[6]. Diagnostic procedures for restaging vary in performance and the way they come to conclusions. It is important to determine down-staging of mediastinal nodes and subsequently for selecting patients for surgery. Restaging is difficult especially when mediastinoscopy has been performed previously. Remediastinoscopy gives inadequate information caused by incomplete procedures in 40% of patients due to fibrosis[7]. PET-CT is by far superior to re-mediastinoscopy[8]. Restaging mediastinal metastases with PET as described in four studies show sensitivities of 50-71% after various induction therapies reflecting the need for pathologic confirmation[9]. A study in 93 patients, restaged after chemoradiotherapy with repeated PET-CT and various biopsy techniques (including EUS) demonstrated a false negative rate of 20% and a false positive rate of 25%. Repeated PET-CT was more accurate than repeated CT for all stages[10].

Endoscopic techniques are of interest because they can be performed repeatedly and deliver material for cytopathologic analysis through transbronchial fine needle aspirations (TBNA). Recently a study was published on restaging with TBNA after induction chemo-radiotherapy[11]. A correct diagnosis was obtained in 71% of patients. Nowadays this technique can be performed under guidance of linear ultrasound bronchoscopes (EBUS-TBNA). This technique is very useful in staging the mediastinum in patients without a prior treatment[12, 13]. A recent study described the experience with EBUS-TBNA in restaging the mediastinum after induction chemotherapy in patients with stage IIIA NSCLC[14]. A sensitivity, specificity, positive and negative predictive value and diagnostic accuracy of 76%, 100%,100%, 20% and 77% respectively were found.

Also endoscopic ultrasound (EUS) is feasible in mediastinal restaging after induction chemotherapy as demonstrated in a study of 19 patients with a reported sensitivity of 75% and accuracy of 83%[15]. This sensitivity is lower than sensitivities reported in the pre-treatment analysis[16]. EUS-FNA for restaging after chemo-radiotherapy has been described in two studies[10, 17][1, 2]. One study described restaging results in 93 patients but did not report data on the accuracy of EUS in this setting[10]. One study in 14 patients reported a diagnostic accuracy of 86%[17].

The paucity of articles describing the use of EUS for restaging might be caused by a reluctance to perform this procedure on patients with a radiation esophagitis.

In this prospective study, both PET-CT and EUS-FNA were used for restaging patients with stage III NSCLC after induction therapy (chemotherapy or concurrent chemo-radiotherapy).

The performance of both tests was compared with those of surgical dissection of the mediastinum.

PATIENTS AND METHODS

Entry Criterion

Between February 2006 and May 2008 patients with stage III NSCLC were included after a written informed consent was obtained.
All patients were initially staged with MRI or CT of the brain and integrated PET-CT. Subsequently the mediastinal nodal status was verified with EUS-FNA in 26 patients, cervical mediastinoscopy in 1 patient and Transbronchial Needle Aspiration in 1 patient. Patients were eligible if the pathologically proven metastatic site determined the disease stage and could be reached with EUS-FNA at restaging.
Pulmonary function tests were performed to estimate operability after induction treatment.

Endoscopic ultrasound and pathologic analysis

EUS was performed with Pentax ultrasound endoscopes with a Hitachi EUB-5500 processor. The procedures were performed under conscious sedation with midazolam and with local anaesthesia that was sprayed in the oropharynx (lidocain 1%) and lidocain gel 20 mg/ml. FNA was performed with Cook Echotip 22G needles. Per nodal site, 3 to 4 passes were performed and at least two aspirates were smeared on slides. The remaining biopsies were suspensed in a conservation medium (Carbowax 2% [polyethyleenglycol 20 g in ethanol 96% methylated and filled up to 1000 ml with water]). If the slides showed no tumour cells, the remaining material was also analysed for tumour cells. If malignant cells were detected on the slides, the suspension in carbowax was used for immunohistochemical analysis to determine the subtype of NSCLC.

Imaging

PET-CT scans were performed on an integrated PET-CT scanner (GE Discovery ST PET-CT scanner; General Electric, Milwaukee, Wis). Patients were asked to fast for 6 hours and than received 4 MBq/Kg body weight (min 252 and max 400 MBq) fluorodeoxyglucose intravenously followed by PET after 1 hour. Serum glucose had to be lower than 10 mmol/L. A reconstruction with CT attenuation correction was performed. An additional contrast enhanced helical CT-scan from head to pelvis was performed immediately after PET-CT scanning.
Ratios of FDG uptake in the lymph nodes and background uptake as measured in the ascending aorta were calculated before and after the initial treatment.
Metabolic response was scored according to criteria proposed by the European Organization for Research and Treatment of Cancer (EORTC)[18]. Progressive metabolic disease (PMD) was classified as an increase of 25% FDG uptake compared to baseline. Stable metabolic disease (SMD) was classified as an increase in FDG uptake of less than 25% or a decrease of less than 15%. Partial metabolic

response (PMR) was classified as a reducion of more than 25%. Complete metabolic response (CMR) was classified as a complete resolution of FDG uptake.
The response was judged as down-staged if there was at least a partial metabolic response in the metastatic lymph node.

Treatments
The patients were treated with various regimen consisting in most cases of concurrent chemo-radiotherapy and only a few patients received chemotherapy without concurrent radiotherapy.
Restaging procedures were performed within 2 weeks after ending the induction regimen and involved at least an integrated PET-CT and EUS-FNA. The same mediastinal lymph node stations found to be positive at initial analysis were approached again for needle aspirations at restaging.
If restaging with EUS-FNA showed no tumor cells, patients were considered as down-staged. Therapy proceeded with a thoracotomy including a lobe specific mediastinal lymph node dissection. During a right thoracotomy lymph node resection was performed for Naruke 4, 7, 10 and 11 for Right Upper Lobe lobectomy[19]. When possible Naruke 2 was resected as well. For the Right Lower Lobe lobectomy Naruke 8, 9, 10, 11, and 7 were taken. As much as possible lymph node tissue was resected en bloc with the lobectomy. The same nodes were resected during left thoracotomies although Naruke 2 and 4 left were not always easy to retrieve due to preoperative treatment alterations.

The study was approved by the local medical ethical committee.

RESULTS

Patient characteristics
Table 1 shows the characteristics of the 28 patients included.
The median number of nodal sites biopsied during initial staging procedures was 3 (range 1-6).
Six patients received chemotherapy and 22 patients concurrent chemo-radiotherapy as induction treatment. In thirteen of the patients who received concurrent chemo-radiotherapy, two courses of full dose chemotherapy preceded chemoradiotherapy.
Restaging EUS-FNA and PET-CT was performed within 2 weeks from last treatment date (median 10 respectively 6 days). In 3 patients the restaging PET-CT was performed in the last week of chemoradiotherapy.
Four patients who were not down-staged were treated with additional radiotherapy with a mean interval of 25 days between the end of treatment and the start of the additional treatment.

Table 1
Patient characteristics (28 patients)

Median age (range) (year)			60 (47-78)
Gender			
Male			22
Female			6
Histology			
Adenocarcinoma			6
Squamous cell carcinoma			15
Nonspecified NSCLC			7
Induction treatment			
Cb/Pac Q3wk 2x			1
G/C Q3wk 2x			4
G/C Q3wk 3x			1
G/Cb Q3wk 2x	followed by	C/D QW 6x + 59,4 Gy	1
G/C Q3wk 2x	followed by	C/D QW 6x + 66 Gy	12
C/D QW 6x + 66 Gy			2
C/D QW 6x + 60 Gy			1
C/D QW 6x + 45 Gy			6
Mean interval between end of treatment and restaging (days)			
Restaging PET			6 (range 4 – 172)
Restaging EUS			10 (range 6 – 196)
Distribution of initial N2-disease			
4L			12
7			12
4L + 7			2
8			1
5			1

NSCLC, non-small cell lung cancer, no histologic classification available at initial analysis
Cb/Pac, carboplatin AUC 6 day 1 + paclitaxel 175 mg/m2 day 1; G/C, gemcitabin 1250 mg/m2 day 1,8 + cisplatin 80 mg/m2 day 1; C/D, cisplatin 20 mg/m2 day 1 + docetaxel 20 mg/m2 day 1; Gy,Gray; EUS, endoscopic ultrasound; PET, positron emission tomography.

EUS-FNA of mediastinal nodes at restaging.
During restaging a median number of 2 (range 1-3) nodal sites were approached with EUS-FNA. There were no complications of EUS-FNA procedures. Particularly, pain during the procedure was not mentioned by the patients. Fifteen out of 28 patients had a restaging EUS-FNA that showed no tumor cells in the mediastinal lymph nodes and in one patient pathology was not conclusive. In the other patients tumor cells were present. A thoracotomy was performed in 13 out of the 15 down-staged patients and in the patient with inconclusive pathologic results. Two patients who were down-staged were ultimately considered not operable. One patient because of a necrotizing reaction in the radiated tumor with probable secondary infection (recovering well after antibiotics) and the other patient was not operable due to insufficient pulmonary function tests. In one down-staged patient a left-sided pneumonectomy was performed but a lymph node dissection was omitted The predictive value of a negative restaging EUS for negative findings at surgical evaluation of resected nodes was 91.6 %.
One patient had a lobectomy of the left lower lobe in spite of a tumor-positive restaging EUS-FNA but an almost complete diminished FDG-uptake. However, pathology confirmed small number of tumor cells in a mediastinal lymph node. The accuracy of EUS-FNA in restaging was 92.3%.

FDG-PET response in mediastinal nodes at restaging.
Table 2 shows the results of nodal restaging with FDG-PET. There were 7 patients with a complete metabolic response (CMR). Two of these patients had persistent nodal disease at restaging EUS and so were not operated according to the protocol. Four patients had pathologic down-staging confirmed with surgery. For one patient with a CMR there was no surgical verification of the nodal status because a lymph node resection was not performed during thoracotomy.
Eighteen patients showed a partial metabolic response (PMR) of nodal disease with FDG activity decreases ranging from 26% to 98%.
In this group 7 patients were tumor positive with EUS-FNA and their FDG activity decrease was between 42% and 98%. One patient was operated although the EUS-FNA revealed persistant nodal disease and the nodal status was verified during operation.
In 7 patients with PMR of the nodes, down-staging was confirmed with surgery. One patient had persistent nodal disease although the EUS-FNA showed no tumour anymore. For 2 patients PMR could not be verified. One patient had a bad general condition due to infection in a necrotic irradiated lung and one patient was in good condition but had insufficient pulmonary function tests.
Two patients showed stable metabolic disease (SMD) matching persistent metastatic nodal disease with restaging EUS-FNA. Two patients had progressive metabolic disease (PMD). In one of them a thoracotomy confirmed pathologic down-staging as was suggested by EUS-FNA results. One patient demonstrated persistent nodal disease with EUS-FNA and so was not operated.

Table 2
Results of restaging evaluation and surgery of nodal disease

Case No	Positive Naruke stations at initial analysis	Restaging EUS result	Restaging PET result		Further treatment	Surgical restaging
			Decrease FDG (%)	Metabolic Response		
1	4L	Negative	103	CMR	Pneumonectomy L	NA
2	7	Positive	76	PMR	RT	NA
3	7	Negative	-69	PMD	Pneumonectomy L	Negative
4	7	Positive	98	PMR	Lobectomy LLL	Positive
5	4L	Positive	76	PMR	CTRT	NA
6	5	Negative	26	PMR	Lobectomy LUL	Negative
7	8	Negative	103	CMR	Lobectomy LUL	Negative
8	7	Negative	95	PMR	Pneumonectomy R	Negative
9	4L + 7	Positive	-73	PMD	None	NA
10	4L	Inconclusive	90	PMR	Pneumonectomy L	Negative
11	4L	Negative	200	CMR	Pneumonectomy L	Negative
12	4L + 7	Negative	91	PMR	Pneumonectomy L	Negative
13	4L	Negative	94	PMR	Antibiotics	NA
14	7	Positive	3	SMD	CTRT	NA
15	4L	Positive	-1	SMD	WBRT	NA
16	7	Negative	110	CMR	Bilobectomy	Negative
17	4L	Negative	89	PMR	Lobectomy LLL	Negative
18	4L	Negative	91	PMR	Pneumonectomy L	Negative
19	4L	Positive	74	PMR	CT	NA
20	7	Positive	72	PMR	Thoracic RT	NA
21	7	Negative	108	CMR	Pneumonectomy R	Negative
22	7	Negative	97	PMR	None	NA
23	4L	Negative	40	PMR	Nodal dissection*	Positive
24	4L	Positive	113	CMR	Immunotherapy	NA
25	7	Positive	78	PMR	Immunotherapy	NA
26	7	Positive	113	CMR	RT metastasis	NA
27	4L	Positive	42	PMR	None	NA
28	7	Negative	98	PMR	Pneumonectomy L	Negative

CMR, complete metabolic response; PMR, partial metabolic response; PMD, progressive metabolic disease; SMD, stable metabolic disease; L, left side; R, right side; LLL, left lower lobe; LUL, left upper lobe; NA, not available; WBRT, whole brain radiotherapy; CTRT, concurrent chemo-radiotherapy; CT, chemotherapy; EUS, endoscopic ultrasound; PET, positron emission tomography
* Patient had a local mediastinal recurrence 2 years after a lobectomy left lower lobe

Relationship between pathologic and metabolic down-staging

The relationship between pathologic down-staging and metabolic downstaging is shown in table 3.

As all but one of the 12 patients with positive EUS-FNA at restaging were not operated according to protocol, verification of the metabolic results was not possible. In this group of cytologically non-down-staged patients, metabolic down-staging was observed in 9 subjects. One patient was operated although restaging EUS-FNA revealed persistent nodal disease. The decision for this was influenced by the convincing (partial) metabolic response this patient showed, Surgery verified the absence of pathologic down-staging.

In 3 out of 14 patients with surgically verified restaging, the FDG-PET scan did not predict the malignant status of the nodes (table 2).

Table 3

Relation between down-staging with EUS-FNA and FDG-PET

	No metabolic down-staging by PET (PMD and SMD)	Metabolic down-staging by PET (CMR and PMR)
Down-staging negative (vital cells)	3	9
Down-staging positive (no vital tumor cells)	1	15*

EUS-FNA, endoscopic ultrasound-fine needle aspiration; FDG-PET, 18F-fluorodeoxyglucose- positron emission tomography; PMD, progressive metabolic disease; SMD, stable metabolic disease; CMR, complete metabolic response; PMR, partial metabolic response.

* One patient had inconclusive cytology and a PMR of the mediastinal nodes.

FDG-PET response in the primary tumor.

The decrease in metabolic activity in the primary tumor in relation to the pathologic findings at surgery is demonstrated in table 4. One patient without metabolic down-staging of the primary tumor was operated and pathologic analysis revealed vital tumor. Eleven patients treated with surgery showed decreases in metabolic rate regarded as PMR. In this group 6 patients showed a complete pathologic response (including one necrotizing tumour) and 5 still had vital cancer tissue in the resected specimen.

There was one patient with a CMR who still had vital tissue in the resected specimen of the primary tumor.

Table 4
Relation of metabolic response and pathologic results at thoracotomy for the primary tumor

case nr	Restaging FDG-PET results		Operation	Pathology
	Decrease FDG uptake(%)	Metabolic response		
1	96	PMR	Pneumonectomy L	CR
3	73	PMR	Pneumonectomy L	CR
4	99	PMR	Lobectomy LLL	Vital tumor + N2 metastasis (N8/9)
6	14	SMD	Lobectomy LUL	Vital tumor + N1 metastasis
7	74	PMR	Lobectomy LUL	Vital tumor + N1 metastasis
8	87	PMR	Pneumonectomy R	Vital tumor; no node metastasis
10	85	PMR	Pneumonectomy L	CR
11	86	PMR	Pneumonectomy L	Necrotizing tumor; no node metastasis
12	48	PMR	Pneumonectomy L	Vital tumor + N1 metastasis
16	77	PMR	Bilobectomy R	Vital tumor
17	101	CMR	Lobectomy LLL	CR
18	101	CMR	Pneumonectomy L	Vital tumor; no node metastasis
21	93	PMR	Pneumonectomy R	CR
28	97	PMR	Pneumonectomy L	CR

FDG-PET, 18F-fluorodeoxyglucose- positron emission tomography; PMR, partial metabolic response; SMD, stable metabolic disease; CMR, complete metabolic response; CR, complete pathological response

Relationship between metabolic responses in primary tumor and lymph nodes
When metabolic responses are devided in metabolic down-staging (CMR+PMR) and metabolic non-down-staging (SMD+PMD), the metabolic staging results matched for nodal disease and primary tumour in 24 patients. In 3 patients, the metabolic staging results did not match and for one patient the relation can not be studied because the primary tumour was resected 2 years before.

Metabolic response in relation to treatment
In 6 patients treated with chemotherapy, PMR was observed in the nodes of 5 patients and SMD in 1 patient (metabolic downstaging in 83%). In 22 patients treated with chemo-radiotherapy there were 12 patients with PMR and 7 with CMR in the lymph nodes (86% with metabolic downstaging).
The metabolic responses after chemotherapy in the primary tumor were classified as PMR in 4 patients, PMD in 1 patient and SMD in 1 patient (metabolic downstaging in 67%). The metabolic response in 21 primary tumors treated with chemo-radiotherapy were classified as PMR in 16 patients, CMR in 2 patients, PMD in 2

patients and SMD in 1 patient (metabolic downstaging in 86%). One patient had a resection of the primary tumor two years before.

Follow up

In figures 1 and 2, Kaplan Meier curves show overall survival and progression free survival respectively for down-staged patients and patients with persistent nodal disease . The down-staged patients had a median follow up time of 11.1.months (range 1.6 – 32.6.months). The patients with persistent nodal disease had a median follow up time of 4.2 months (range 1.0 – 29.5 months).

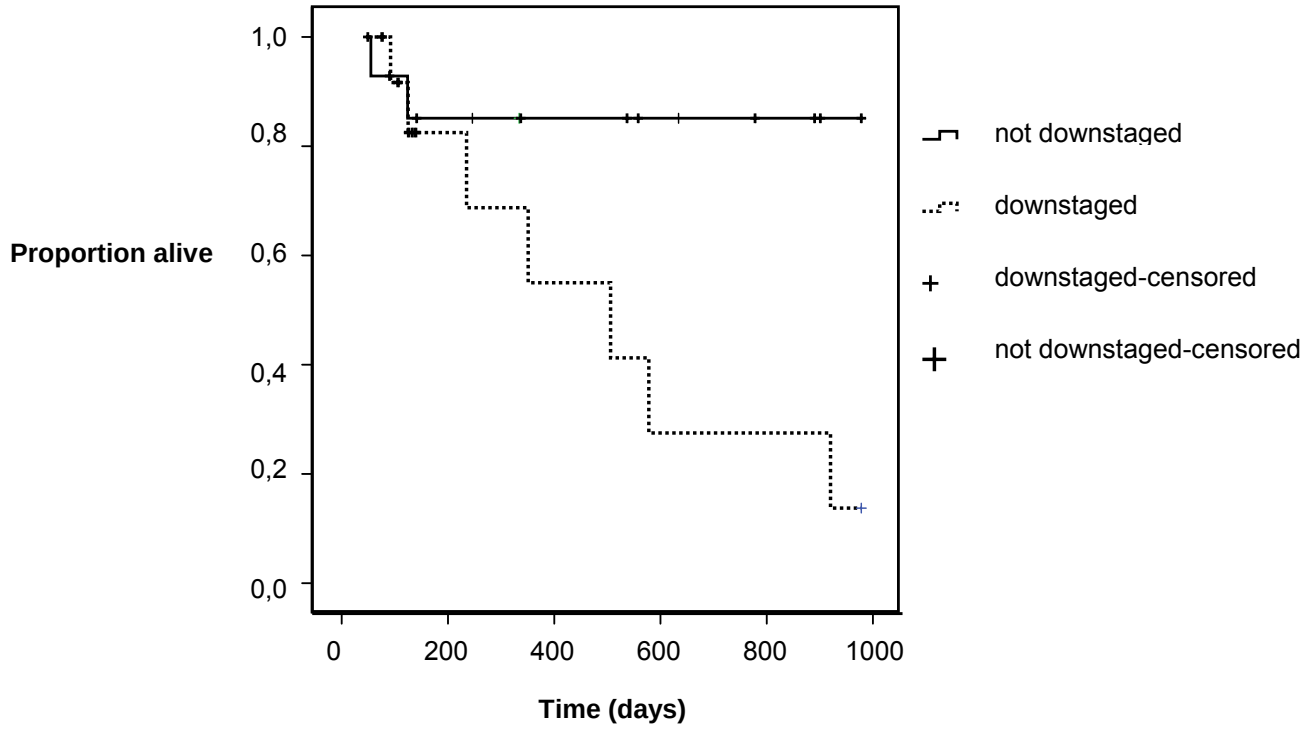

Figure 1. Kaplan-Meier curve of overall survival for patients with stage III non-small cell lung cancer after treatment with chemo(radio)therapy.
Patients with or without down-staging at repeated endoscopic ultrasound guided fine-needle aspirations or surgical exploration of mediastinal lymph node metastasis are depicted. (P=0.049). Follow-up time not downstaged (days) = 139(76-978); follow-up time downstaged (days) = 337(49-978).

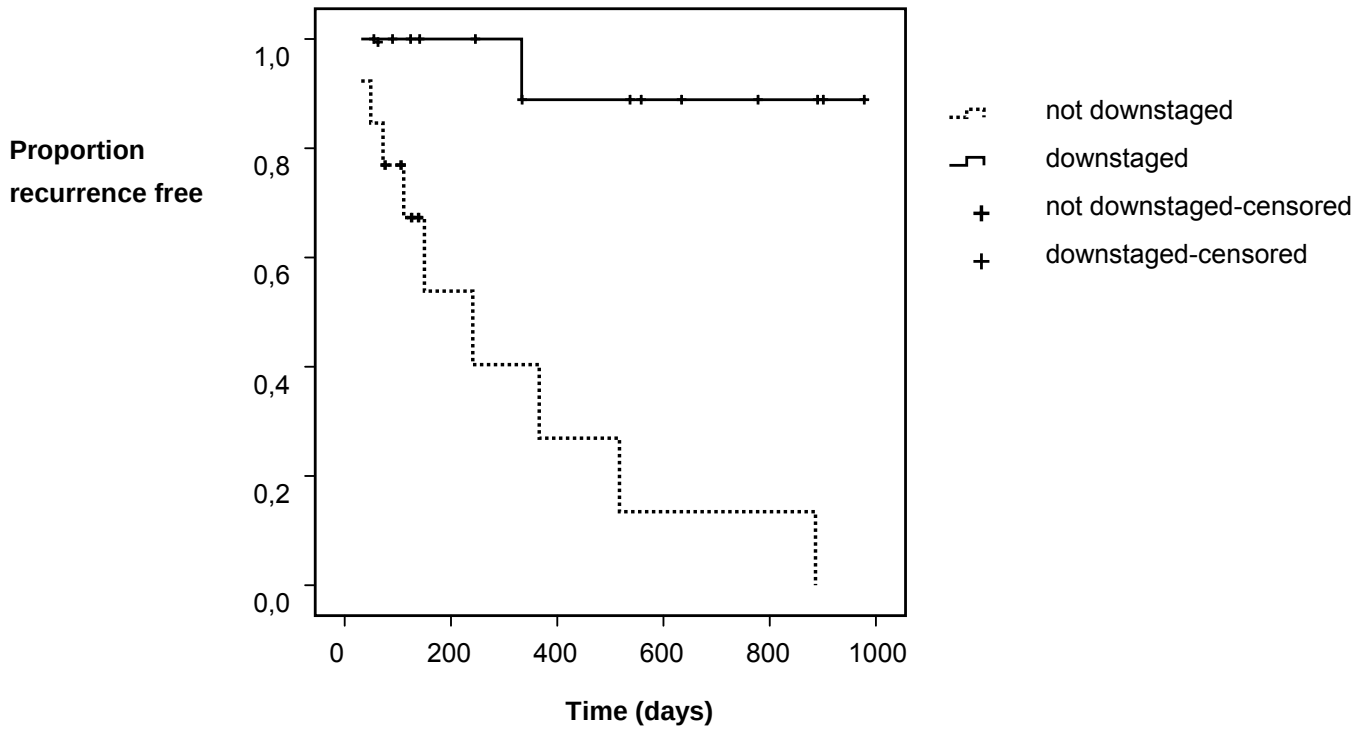

Figure 2. Kaplan-Meier curve of recurrence free survival for patients with stage III non-small cell lung cancer after treatment with chemo(radio)therapy.
Patients with or without down-staging at repeated endoscopic ultrasound guided fine-needle aspirations or surgical exploration of mediastinal lymph node metastasis are depicted. (P<0.0002). Follow-up time not downstaged (days) = 126(31-886); follow-up time downstaged (days) = 334(49-978).

In the group of 13 down-staged patients with surgical confirmation, 2 patients died. One suffered a post-pneumectomy syndrome after a right sided pneumonectomy and the other died of a pneumonia 3 months after a left pneumonectomy. Ten patients are alive without signs of tumor activity and one patient had a local recurrence. The two down-staged patient who were not operated did not show local recurrence or metastatic disease for 3 and 24 months after treatment.
In the only patient operated in spite of a positive restaging EUS-FNA, a local recurrence was detected after 29 months of follow up. Of the remaining 11 patients with a positive restaging EUS-FNA, 7 developed metastatic disease and 1 developed local recurrence during follow up.

DISCUSSION

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is a mediastinal staging modality that has been developed in the last decade. High sensitivities and specificities have been reported in a large number of studies and in a recently reported meta-analysis[16]. Since resolution of ultrasound techniques is high and fine needle aspirations are performed in real-time, EUS and EBUS or combinations of both with FNA have higher diagnostic accuracies than blind TBNA and PET-CT. Therefore they will become the preferred restaging tool in near future.
There are only two studies that compared pathologic findings in material obtained with EUS or EBUS with changes in FDG uptake in restaging NSCLC patients after induction chemoradiotherapy[10, 11]. In one study patients restaged with TBNA after induction chemoradiotherapy showed false-positive PET-CT results in 5 out of 11 patients. All five patients with a positive TBNA had a positive PET-CT result and the two negative PET-CT scans were truly negative[11]. A study in 93 patients demonstrated a diagnostic accuracy of PET-CT at restaging of 92% for stage 0 and 89% for stage I but only 69% for persistent stage III disease[10].
The predictive value of downstaging after induction therapy for stage III nodal disease is generally recognized. Patients are selected for surgical treatment based on restaging results although the role of surgery in stage III NSCLC after chemoradiotherapy is still under debate. Evaluation exclusively on PET-CT imaging is not reliable enough for evaluation of the mediastinum, therefore pathologic confirmation is warranted[9].
Once a mediastinoscopy is performed during the initial diagnostic analysis, a remediastinoscopy should not be performed[8][3]. At the initial staging endoscopy is the procedure of choice in case a restaging after induction therapy is planned.
Our study confirmed that restaging with EUS-FNA after induction chemotherapy or chemoradiotherapy is feasible. Twenty-two of the studied patients underwent EUS-FNA after induction with concurrent chemoradiotherapy. There were no complications and no significant complaints of pain although most patients suffered various grades of radiation esophagitis.
This study demonstrated that a negative EUS-FNA at restaging predicted down-staging very well. In only one of the 14 patients with surgical confirmation of the N status the EUS-FNA result at restaging was false negative. A negative predictive value of 91.6% and a diagnostic accuracy of 92.3% were calculated.
A recent study on 124 patients treated with induction chemotherapy– not chemoradiotherapy - for stage IIIA nodal disease reported that EBUS-TBNA is a sensitive, specific, accurate, and minimally invasive test for mediastinal restaging of patients with NSCLC. However, because of the low negative predictive value, tumor-negative findings should be confirmed by surgical staging before thoracotomy. Our results on EUS-FNA are in line with EBUS-FNA with the exception of our high negative predictive value of 91.6%. False negative findings are a result of the vanishing vital tumor mass. Nests of vital tumor cells remain which can be missed by passes of fine needle aspirations. EUS-FNA seem to perform better in restaging after chemo-radiotherapy than blind and ultrasound guided TBNA’s.

The bigger tumor samples obtained with EUS-FNA or the lower pretest probability of residual tumor in the mediastinal nodes may be an explaination for this observation The patients undergo EUS very easy and in a quiet setting. Nodes can be identified very accurate due to obvious anatomical landmarks and a non-moving patient. To minimize the chance of missing vital tumor parts in nodal tissue, we try to pass the needle under suction at least four times per site and in different directions in the node. However, in some cases the initially positive nodes disappeared completely or turned out to be haze, hardly recognizable structures. In three cases the lymph nodes were rubbery structures and hard to biopsy.
During EBUS the patients are coughing and are restless even with midazolam. The US image is moving accordingly. The patients are generally too restless to sustain a long procedure with many biopsies at different sites. Orientation is more difficult because of less evident landmark structures. These characteristics may account for the worse perfomance of EBUS in restaging studies.
FDG-PET was performed in every patient before start of treatment and again at the end of induction treatment at the same time as restaging EUS. Metabolic down-staging in 75% of patients with persistent nodal disease at restaging EUS-FNA was seen (table 3). When EORTC criteria for metabolic response are used, pathologic verification is required. The criteria,as proposed by the EORTC for stable or progressive metabolic disease (defined as a decrease of less than 25% or an increase in FDG uptake), predicted the post-treatment lymph node state better but still require pathologic confirmation.
Based on receiver operating characteristic (ROC) curves, the optimal time to perform a repeat PET-scan to predict the pathologic stage after chemo-radiotherapy, is about 1 month. This conclusion was derived from a retrospective analysis of 93 patients who received at least 60Gy of radiotherapy[20]. In our study the interval between end of treatment and restaging PET-scan was much shorter (median of 6 days) which might have influenced the accuracy. Our timing derived from a treatment strategy proposed for the most early patients in the study. These patients were treated with 45 Gy of concurrent chemo-radiotherapy. After restaging, the patients were operated when they were down-staged but proceeded with radiotherapy until 66Gy in case they had persistent nodal disease. To minimize the interruption of radiotherapy, the restaging tests were performed immediately after chemoradiotherapy. During the study the treatment strategy was changed and patients were offered 66 Gy of radiotherapy in a concurrent setting. At the end of treatment the patient were restaged with the same short interval as the former patients.
Based on recent information, nowadays patients undergo restaging investigations with an interval of 1 month.
A study reporting on the performance of repeated PET-scans after neoadjuvant chemo-radiotherapy, showed that a decrease of 50% of the maximum standardized uptake value (SUV) in the nodes was highly likely for down-staging with a likelyhood ratio of 7.9[10].
In this study, 21 patients showed a decrease of FDG-uptake in the nodes of at least 50% but two thirds had no pathologic down-staging.

In our series there was just 1 patient without metabolic down-staging in the lymph node who had pathologic down-staging with EUS-FNA, verified by surgery. Local inflammatory reactions to radiotherapy in and surrounding the tumor can account for increased FDG uptake. This effect can influence data of respons when restaging PET-CT is performed shortly after radiotherapy as in this study. Regarding the decreases of FDG uptake in the nodal metastases for down-staged as well as non-down-staged patients in our series, this effect seems to have little influence. The metabolic response in the nodes overestimates the pathologic response in this study.
Whether surgery should be the consequence of mediastinal down-staging after induction treatment with chemo-radiotherapy is still controversial. As demonstrated in table 4,in just over half the number of cases remnants of vital tissue, recognizable as tumor tissue, were seen in the primary tumor and hilar lymph nodes. These remnants could still represent vital tumor parts able to grow out to local recurrences or dying tumor cells on their way to necrosis and disappearance.
Decreases of maximum SUV in repeated PET-CT scans can predict the effects of chemo-radiotherapy on the primary tumor. It is highly likely that a patient has a complete pathological response when the maximum SUV decreases by at least 75% or a partial response (nodal downstaging but no complete pathological response) when the maximum SUV decreased 55% or more[10].
In this study 6 patients had a complete pathological response of the primary tumor and in 5 of them the FDG uptake decreased by more than 75%. There was 1 patient with a decrease of 73% of FDG uptake and a complete pathological remission..
In two thirds of patients with a partial pathologic response, the decrease of FDG uptake was more than 55%. The decreases in FDG uptake in these primary tumours varied even from 74% to 101%.
It seems that treatment affects the extend of the metabolic response. Although the metabolic responses in the lymph nodes were comparable in patients treated with chemotherapy and chemo-radiotherapy, complete metabolic responses were only observed in the chemo-radiotherapy group. Metabolic downstaging of the primary tumor was observed more often in patients treated with chemo-radiotherapy and complete metabolic responses were only observed in the patients treated with chemo-radiotherapy. These observations might reflect the more aggressive local approach with chemo-radiotherapy.
Based on metabolic data it is impossible to discriminate between complete pathologic remission or persisting vital parts in the primary tumor. Decisions of surgery or not can not be safely made on decreases in FDG uptake.
The effects of the short interval between last radiotherapy dose and restaging PET-CT as in this study seem to be limited .High decreases of FDG uptake were observed in the patients with a complete pathologic response as well as in the patients with a partial pathologic response .
Studies have demonstated that tumor progression is more likely to happen at distant sites than locally[3, 5, 6]. For this moment it is still not proven that the more aggressive additional local therapy, as surgery is, will improve survival rate.
The prognostic significance of nodal down-staging is illustrated in this study. The overall survival curve of the downstaged patients shows an initial drop caused by

postoperative mortality. The divergence of the curves of down-staged patients versus patients with persistent nodal disease is obvious but the follow up is still very short. The right sided paratracheal nodes can not be reached with EUS. This part of the mediastinum could be covered by EBUS. A study on the accuracy of EBUS as restaging tool after only induction chemotherapy reported a very low negative predictive value of 20%[14] and studies with EBUS-TBNA after induction chemo-radiotherapy have to be awaited. It can be expected that EBUS-TBNA will be superior in this respect in comparison to blind TBNA as was demonstrated in patients who were not pre-treated[21, 22].

CONCLUSION

In conclusion, EUS-FNA is very well tolerated in patients treated with induction chemo-(radio)therapy and negative cytologic results predict down-staging very well. The value of PET scanning in restaging is limited because metabolic responses scored with EORTC criteria do need pathologic confirmation.

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Chapter 3

Diagnosing Infectious Spondylodiscitis with Endoscopic Ultrasound

J Bronchol Intervent Pulmonol 2012;19:82-4

Jos A. Stigt, Maurice J. Wolfhagen, Martijn F. Boomsma,
Adriaan K. Mostert, Harry J. M. Groen

INTRODUCTION

In the workup of infectious spondylodiscitis (ISD), blood cultures and computed tomography (CT)-guided biopsies or open biopsies are performed to verify the diagnosis and isolate pathogenic microorganisms. Herein we report the use of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) to diagnose ISD.

CASE REPORTS

Patient 1

A 64-year old man was analysed for fatigue, weight loss and back pain between his shoulders. He had no fever. He had a history of myocardial infarction one year before followed by 2 percutaneous transluminal angioplasty procedures 1 year and 5 months before disease symptoms. Blood chemistry showed an elevated C-reactive protein (CRP) of 64 mg/L but no other abnormalities.
A CT scan, ordered to rule out pulmonary embolism, showed no pulmonary embolus but a prevertebral mass with destruction of the ventral part of the endplates diagnostic for spondylodiscitis (Fig. 1).
EUS-FNA showed an active inflammation with necrosis but no malignancy. Microbiological culture showed *Staphylococcus aureus (S.aureus)* susceptible to methicillin and flucloxacillin.
The patient recovered after 6 weeks of intravenous flucloxacillin.

Figure 1: The left panel shows a sagittal scan demonstrating classical extensive destruction of the ventral part of the endplates. A prevertebral mass that extended from Th4 up to Th7 is continuous with the intervertebral disc space at the level of Th5-Th6. The right panel shows an axial scan of the prevertebral mass.

Patient 2

A 76-year old man had 3 acute admissions in 4 weeks for heart failure with atrial fibrillation. He had a history of chronic obstructive pulmonary disease (COPD) and longer existing back pain. There was no fever, physical examination was unremarkable. During the last admission an elevated CRP of 262 mg/L was noted for the first time. Abnormalities on standard chest radiography were suspect for malignancy. A CT of the chest without contrast (due to diminished renal function) revealed a prevertebral mass (Fig. 2).

The aspirate obtained with EUS-FNA had a purulent aspect, with abundant grape-like cocci at cytology. Culture confirmed *S. aureus* susceptible to methicillin, penicilline, amoxicillin, and clindamycin.

The patient recovered with amoxicillin administered intravenously for 2 weeks and subsequently clindamycin orally for 4 weeks. Endocarditis or skin infections were excluded as possible source of haematogenous spread. During treatment a persisting elevated erythrocyte sedimentation rate was analysed resulting in the diagnosis of multiple myeloma.

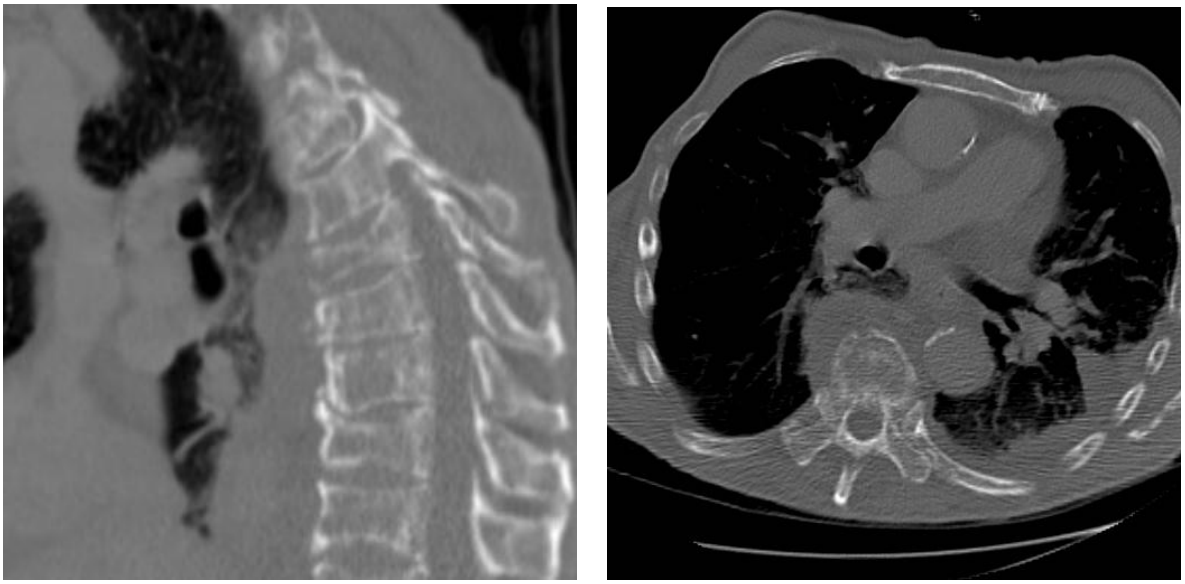

Figure 2: A sagittal scan shows a prevertebral mass. Note the preservation of the posterior elements, including facet joints, lamina, and spinous process in both cases. The mainly right-sided prevertebral mass is shown on an axial scan on the lower panel. The mass is in close contact with the collapsed and eroded vertebra Th8. There is a small region of ventrally located cortical bone destruction in Th8.

COMMENTS

We have encountered 2 patients in whom ISD was diagnosed with needle aspiration under EUS guidance.
ISD is an infection of the intervertebral disc space often with involvement of the adjacent vertebral bodies. The incidence is low and estimated at 2.4 per 100,000 inhabitants and increases with age. The thoracic spine is affected in 30% of patients and the lumbar spine in 60%.
The clinical course is often insidious with back pain as a predominant symptom. Fever is not always present and biochemical tests are non-specific [1]. Both our patients had no fever and were in fact referred because of a suspicion for malignancy.
Hematogenous spread of bacteria from infections elsewhere in the body is the main cause of ISD. Sometimes ISD is a complication of local invasive spinal procedures or contiguous spread from adjacent tissue infection. In patient 1 probably repeated angiographic interventions initiated infection. The risk for developing ISD is affected by predisposing underlying conditions like diabetes, alcoholism or human immunodeficiency virus. The bad general condition and immune compromised state (multiple myeloma) of patient 2 possibly facilitated the development of ISD.
The most sensitive and specific imaging test for spondylodiscitis is magnetic resonance imaging (MRI). T1-weighted images show narrowing of the disc space and low signals consistent with edema in the marrow of adjacent vertebral bodies. T2-weighted images show increased signals in both the disc space and the surrounding vertebral bodies. MRI is very useful in helping distinguish between tuberculous and pyogenic causes and neoplasia. Use of CT scanning adds to MRI, as it better discriminates between bone and soft tissue than MRI[2].
Although imaging helps in the differential diagnosis and is essential for localising the source of infection, verification of the primary process should be performed with biopsy and microbiological cultures. A microbiological diagnosis can be made with blood cultures (positive in about half the patients), CT-guided percutaneous biopsies are performed when blood cultures remain negative or when tuberculosis or malignancy is suspected. Positive cultures with known bacteria are identified in 60-70% of patients with ISD. As in both our patients, *S. aureus,* is the most common causative microorganism accounting for 15-84% of non-tuberculous cases[3]. If repeated percutaneous biopsies do not provide a diagnosis or when the infection is not accessible with a percutaneous approach, a diagnostic yield of 75% is achievable with an open (surgical) procedure.
Next to CT-guided biopsies EUS-FNA provides a more simple, quick and easy to perform procedure to obtain biopsies under direct vision. If lesions are in the vicinity of the esophagus, they can be located with a linear ultrasound device mounted at the top of an endoscope. Biopsy samples or needle aspirations can be derived via the working channel [4].
This is the first report of two cases of ISD diagnosed by EUS-FNA with identification of pathogens. A former case report described EUS-FNA of a paraspinal abscess at the level of C7 and Th1 but no microorganisms were isolated [5].

Although one could argue that the isolated *S. aureus* strains are due to contamination derived from the passage through the throat and esophagus, we think this is very unlikely. First, the high number of colony forming units of *S. aureus* isolated from the samples outnumbering the normal contaminating flora is highly indicative for infection. Furthermore, the direct Gram stains of both aspirates showed Gram-positive cocci. Because of the low sensitivity of the Gram stain one would not expect to find abundant Gram positive cocci in case of contamination.
EUS-FNA is very safe and easy to perform and offers the opportunity to approach vertebral infections from anterior. After cytologic exclusion of malignant causes and isolation of pathogenic microorganisms, an adequate antimicrobial therapy can be started based on culture results.

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Chapter 4

Esophageal fistula after EUS-FNA in a patient treated with Bevacizumab for Non-small Cell Lung Cancer.

Journal of Thoracic Oncology 2013;8:e25-26

Jos A. Stigt, Martijn F. Boomsma, Wouter H. de Vos tot Nederveen Cappel

CASE PRESENTATION:

A 54-years old female patient is presented with a T1aN3M1b, EGFR wild type adenocarcinoma originating in the right lung.
The patient was treated with 4 courses of carboplatin and paclitaxel combined with bevacizumab (15 mg/kg) followed by maintenance bevacizumab.
A partial remission was observed, persisting until after 2 courses of maintenance bevacizumab . At that moment patient exhibited a skin metastasis but target lesions showed no progression and bevacizumab was continued. Since there was doubt if the skin metastasis should be regarded as disease progression, further treatment strategies were considered.
To exclude the presence of an EML-ALK gene reallocation, new samples were required as the original cell blocks were used up. A paraesophageal metastasis as easiest accessible lesion was approached with endoscopic ultrasound (EUS) guided FNA.
A 3rd and 4th bevacizumab course were administered 13 days before, respectively 8 days after EUS. Four weeks after EUS, a CT scan showed a new liver metastasis and increase of brain metastases indicating disease progression. Also a small air configuration in the sampled node was perceived.
In the meanwhile, the patient experienced mild, but gradually progressive retrosternal dyscomfort. Blood chemistry showed elevated C-reactive protein (CRP) of 284 mg/L.
A CT scan (8 weeks after EUS), showed enlargement of the air space in the paraesophageal region. At esophagoscopy, an impression was observed with 2 elongated fissures covered with blood clots. At inspiration, air bubbles escaped from the fissures.
Tube feeding and antibiotics resulted in clinical and biochemical improvement of the patient.
A final CT scan (10 weeks after EUS), showed a progressive air space originating from the paraesophageal mass with access to the esophageal lumen (figure 3).

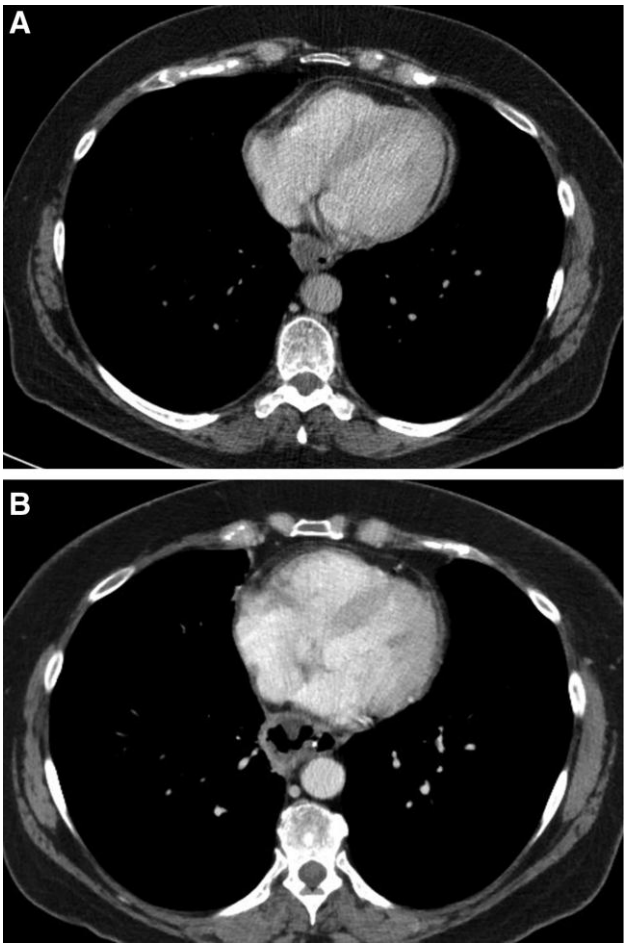
In this patient, a fistula developed between a necrotizing mediastinal mass and the esophagus within 4 weeks after EUS while on bevacizumab treatment. Although cavitating mediastinal nodes were not described in particular previously, formation of bronchoesophageal fistula after bevacizumab was reported before, especially in patients pretreated with (chemo)radiotherapy[1, 2].
In this case there seems a relation between fistula formation and EUS. The high CRP, normalizing during antibiotic treatment, suggests an infectious component. Normally EUS-FNA is a safe technique but infectious mediastinitis in necrotic lymph nodes, resulting in cavitation, was reported previously[3].
In our patient, optimal circumstances for fistula formation were created in the aspirated node by pretreatment with bevacizumab. Repeated needle passes in necrotic tissue caused longitudinal fissures. Delayed wound-healing and secondary infection resulted in fistula formation finally.

EUS-FNA is therefore not recommended in patients on treatment with bevacizumab. If EUS-FNA is nonetheless indicated, antibiotic prophylaxis is advisable.

Figure

Computed tomography (CT) images showing a solid right paraesophageal lymph node metastasis after treatment with 6 courses of bevacizumab and 15 days before endoscopic ultrasound-guided fine needle aspirations (EUS-FNA) (panel A).

The same node 10 weeks after EUS-FNA and after 8 courses of bevacizumab showing extensive cavitation and a breakthrough to the esophageal lumen (panel B).



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Chapter 5

Percutaneous Ultrasound-Guided biopsies in the evaluation of Thoracic Tumours after PET-CT
A prospective diagnostic study

Respiration 2012;83:45-52

Jos A. Stigt, Ad H. Oostdijk, James E. Boers,Jan Willem K van den Berg, Harry J.M. Groen

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SUMMARY

Background.
Lesions detected by PET-CT during the analysis of thoracic tumours are often impalpable at physical examination and subsequent ultrasound (US) may aid in finding these lesions for pathologic evaluation.
Objectives
The success rate of percutaneous US-guided biopsies of palpable and non-palpable lesions and the impact on tumour stage was studied prospectively.
Methods.
Lesions, significant for diagnosis and disease stage, with metabolic activity on PET-CT and presumed appropriate for percutaneous approach under ultrasound guidance were selected for cytologic aspiration or tissue core biopsies.
Results
In 127 patients 134 lesions (subdivided in 24 local thoracic, 74 supraclavicular and 36 distant metastatic lesions) were biopsied percutaneously under ultrasound guidance. Malignancy, benign disease and inadequate biopsies were found in 80%(106/134), 14%(19/134) and 7%(9/134) respectively.
In 55%(56/102) of patients, biopsies confirmed disease stage.
Fifty-one percent (18/35) of distant lesions and 54%(43/68) of supraclavicular lesions were impalpable on physical examination.
Conclusions
US guided biopsies in patients with suspected thoracic malignancy on PET-CT provides an excellent tool for obtaining a pathological diagnosis, leading to a definitive disease stage in over half of the patients.

INTRODUCTION

2-Fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) integrated with computed tomography (CT) provides both anatomic and metabolic information, making it an extremely important tool in characterizing both local thoracic as well as metastatic disease. It has been demonstrated to have a higher diagnostic accuracy than PET or CT alone[1-4]. Therefore it can be considered the most important imaging tool in the analysis of thoracic tumors. Nevertheless, due to the low specificity of PET, pathological confirmation is necessary to establish a definitive diagnosis and/or to provide a tumor stage [5].
While a careful physical examination is considered standard of care, metastatic lesions are often missed and discovered only on PET. The visualization of both palpable and non palpable lesions by ultrasound (US) allows for real-time biopsy. This technique enables safe pathologic sampling with fine needle aspiration (FNA) or tissue core biopsies (TCB) of thoracic tumors in contact with the thoracic cage but also supraclavicular metastatic disease and various sites of distant metastatic disease.
Pulmonologists in Europe have become increasingly familiar with US imaging as endoscopic ultrasound (EUS) and endobronchial ultrasound (EBUS) are nowadays established procedures in the diagnosis and staging of lung cancer. Although US guided percutaneous biopsy and aspiration in the diagnosis of pleural disease are broadly practised by pulmonologists, the technique is seldom applied to other thoracic indications. A recent guideline of the American College of Chest Physicians (ACCP) recommends confirmation of a suspected lung cancer by obtaining tumour cells or tissue with FNA or biopsy if feasible[6]. If pulmonologists become proficient in US guided biopsy they can expand their role in lung cancer diagnosis and staging..
This study is a prospective analysis of the value of percutaneous US guided FNA and TCB in patients with PET-CT suspected thoracic neoplasms as performed by pulmonologists. The evaluating and procedural pulmonologist determined the lesions to be sampled based on their accessibility and visibility by US guided biopsy and their importance in diagnosis and/or staging. It was hypothesized that US accessible lesions (no matter if palpable or not) detected on PET-CT will provide a definitive pathological diagnosis and pathological proof of disease stage.

PATIENTS AND METHODS

Study population

Patients that were selected for US guided sampling all had undergone PET-CT scans for suspected thoracic tumours. Selection was discussed in meetings of nuclear physician and pulmonologist and was based on PET-CT results.
The lesions observed on PET-CT, that were approached for pathologic US guided sampling, had to fulfill three criteria :
First they were judged by a pulmonologist to be accessible by US guided FNA or biopsies (for instance adjacent to the thoracic wall or located in the supraclavicular or axillary region).

In the second place only lesions with increased FDG uptake were selected.
In the third place the lesions had to be significant for tumour staging. Preferably the obtained material delivered a pathological diagnosis and stage verification at once. If PET-CT showed obvious and extensive metastatic disease there were occasional deviations from this principle. In these cases the easiest accessible lesions were approached (for example, some widespread metastasized stage IV tumours were diagnosed according to this strategy with supraclavicular aspirations).
The diagnostic strategy was based on recommendations in the guidelines on the initial diagnosis of lung cancer of the ACCP.
According to legal criteria for medical research our Institutional Review Board stated that approval was not necessary for this study.

Methods
PET-CT scans were performed on an integrated PET-CT scanner (GE Discovery ST PET-CT scanner; General Electric, Milwaukee, Wis).
Percutaneous US guided FNA was performed by a pulmonologist with a Hitachi probe (EUP-C532) with a EZU-PA532 Hitachi Biopsy attachment and a Hitachi EUB-5500 processor. TCB and FNA was obtained under direct vision.
Needles used were Spinocan 0,9 x 88 mm / 20G x 8 cm for cytology. For histologic biopsies Bard core tissue biopsy needles 14G x 13 cm. were used with a Bard magnum biopsy instrument (figure 1).
A local physical examination was performed, before the application of US, to determine if lesions were palpable or not.
All biopsies and aspirations were performed under local anaesthesia of the skin with lidocaine 2%. For local thoracic tumours, specimen were preferentially obtained with core tissue biopsy needles for histologic analysis. Two or 3 core tissue biopsies were taken as a rule for histologic sampling. Supraclavicular and distant metastatic lesions were usually approached with FNA. Specimen obtained with FNA were smeared on slides for morphologic analysis and deposited in fixative for immunohistochemical staining in cases were subtyping was necessary. Two to 4 needle passes into each lesion were taken. There was no on-site evaluation of the samples by a pathologist.
The numbers of adequate (enough tissue or material to make a diagnosis as assessed by a pathologist after microscopy) and inadequate biopsies (not enough tissue or material or not accessible tumour or lymph node by US) were counted. In case of inadequate aspirates or biopsies, CT guided biopsies, endoscopic biopsies or surgical procedures were performed. When these additional investigations had to be performed this was considered as a failure of the US procedure. In case such an approach was not feasible clinical follow-up data were available to substantiate the diagnosis.
Stage categories as defined by the American Joint Committee on Cancer Staging (AJCC)[7] and the sixth edition of the Union Internationale Contre le Cancer (UICC)[8] were applicated.

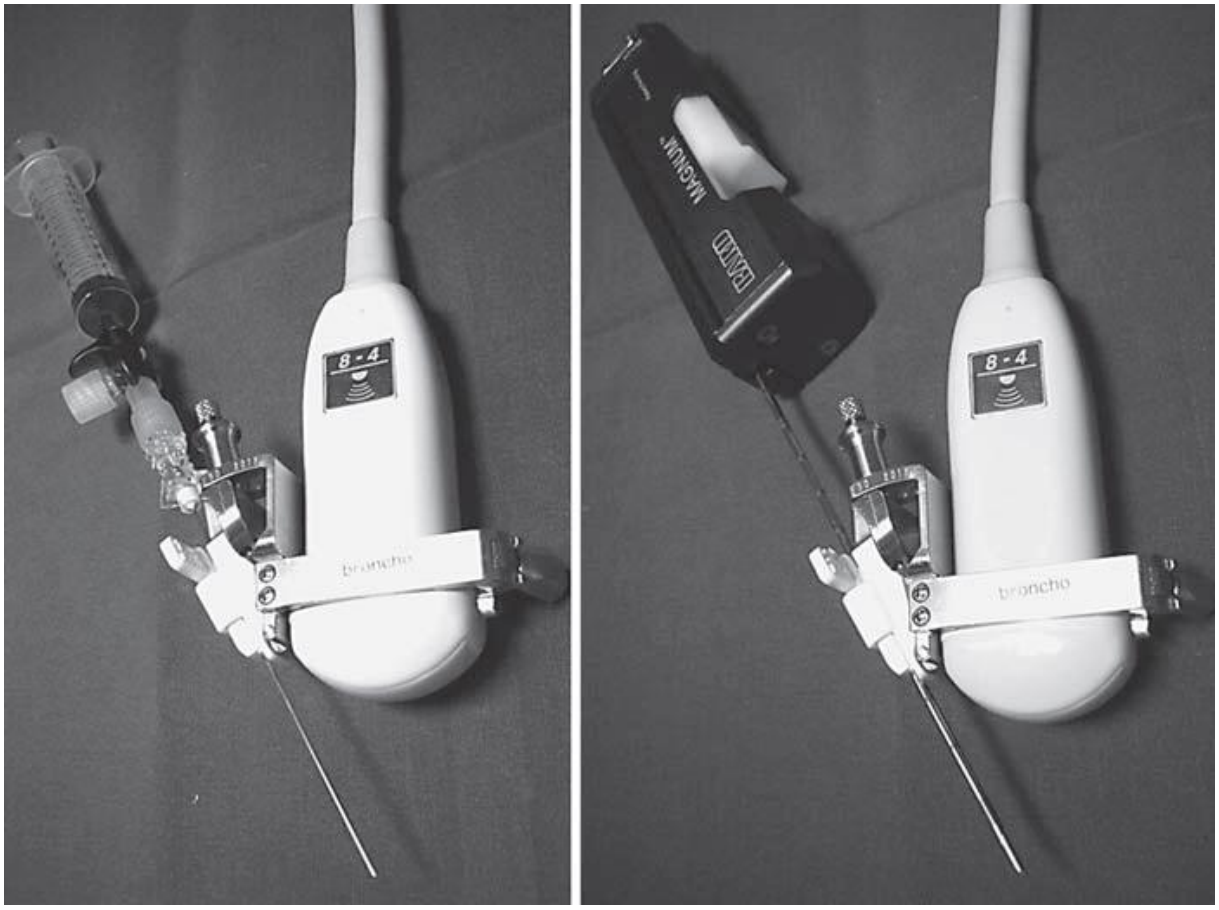

Figure 1 Devices used for ultrasound guided aspirations and core tissue biopsies. Left panel : biopsy attachment with 20G needle mounted on ultrasound probe. Right panel : biopsy attachment with core tissue biopsy needle.

RESULTS

Between March 2006 and May 2009 a total number of 127 patients out of 1056 patients(12%) in whom PET-CT was performed to image suspected thoracic malignancies, were considered suitable for US guided biopsies.
In these patients a total number of 134 lesions were approached for US guided biopsies (FNA, n=107; TCB, n=22; not detectable with US, n=5). In 5 patients US guided biopsies were performed on more than one lesion (one patient had 3 biopsies on different lesions and 4 patients had 2 biopsies on different lesions). Table 1 shows the distribution of FNA and TCB in the subcategories local thoracic, supraclavicular and distant lesions (distant lesions are metastatic lesions other than mediastinal or supraclavicular lesions).
Successful US guided biopsies were performed in 93% of lesions (125/134).

Seven percent (9/134) of US guided biopsies were considered inadequate either because PET-CT observed lesions could not be detected by US (n=5) or biopsies contained inadequate material (n=4) (table 2).

Palpation

At physical examination (performed after PET-CT) palpation of lesions was recorded in 92%(68/74) of patients with supraclavicular lesions and 94%(34/36) of patients with distant lesions. Table 1 shows that 54% of examined supraclavicular and 51% of examined distant lesions were not palpable (as expected, local thoracic disease was not palpable in any of the patients).

Table 1

US guided aspirations and biopsies from PET-CT observed lesions

Location	n	US observed lesions	Lesions not detectable with US	FNA	TCB	Palpation performed	Palpation negative
Local thoracic lesions	24	22 (92)	2 (8)	4	18	NA	NA
Supraclavicular nodes	74	71 (96)	3 (4)	71	0	68 (92)	37 (54)
Distant lesions	36	36 (100)	0	32	4	20 (80)	18 (51)
Total	134	129 (96)	5 (4)	107	22		

Figures in parentheses are percentages.

US, ultrasound; PET-CT, positron emission tomography integrated with computed tomography; FNA, fine-needle aspiration; TCB, tissue core biopsy; NA, not applicable

Table 2
Pathologic results of US guided aspirations and biopsies from PET-CT observed lesions

Location	n	Inadequate biopsies* (%)	Benign diagnosis (%)	Malignant diagnosis (%)	Stage defining lesions (%)**
Local thoracic lesions	24	5 (21)	2 (8)	17 (71)	9 (53)
Supraclavicular nodes	74	3 (4)	7 (9)	64 (86)	32 (50)
Distant lesions	36	1 (3)	10 (28)	25 (69)	20 (80)
Total	134	9 (7)	19 (14)	106‡ (80)	61 (58)

* Inadequate biopsies are failures to find PET-CT observed lesions by US and biopsies without adequate material
as assessed by the pathologist.
** Percentage of malignant lesions that determine the tumour stage.
‡ 106 lesions containing malignant tissue were biopsied in 102 patients. In 4 patients, 2 malignant lesions were biopsied.

Malignancy by US guided biopsies
Overall, malignancy was confirmed in 80% (106/134) of US guided biopsies in PET-CT positive lesions with a predominant representation of NSCLC (51% (68/134)) as shown in table 3. Subtypes of NSCLC in FNA and TCB are shown in table 4. In 3 FNA and 1 TCB subtyping was not possible and in 2 FNA subtyping was not carried out because a histological diagnosis was already available. Local thoracic US guided biopsies were positive for malignancy in 71% (17/24) of lesions, supraclavicular US guided biopsies in 86% (64/74) of lesions and distant US guided biopsies in 69% (25/36) of lesions (table 2). Positive predictive values to find malignancy by US guided punctures in thoracic, supraclavicular and distant lesions observed with PET-CT were 77%, 90% and 69%, respectively.

Benign disease
Benign diagnoses were obtained in 14%(19/134) of PET-CT positive lesions. In 8% (2/24) of local thoracic lesions a benign diagnosis was derived. Supraclavicular US guided biopsies showed benign disease in 10%(7/74) of biopsied lesions and distant US guided biopsies showed benign disease in 28%(10/36) of lesions. Benign diagnoses are listed in table 5.

Table 3
Ultrasound guided aspirations and biopsies from 106 PET-CT observed lesions in 102 patients that lead to pathologically confirmed malignant diagnoses

	Local thoracic lesions	Supraclavicular nodes	Distant lesions
NSCLC	7	45	16
SCLC	0	13	2
LCNEC	1	2	1
Sarcoma	1	2	0
Mesothelioma	5	0	1
Non-pulmonary metastasis	1	1	0
Malignant Lymphoma	0	1	5
Thymoma	2	0	0
Total	17	64	25

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; LCNEC, large cell neuroendocrine cancer

Inadequate biopsies
The overall rate of inadequate US guided biopsies was 7% (9/134) as demonstated in table 2. Inadequate US guided biopsies were performed in 21%(5/24) of local lesions. One patient had a clinical follow-up of local pleural broadening after pneumonectomy during 18 months without any sign of progression. Two lesions were biopsied subsequently by surgery (1 mesothelioma and 1 malignant lymphoma), 1 lesion was biopsied under CT guidance (bronchoalveolar carcinoma). In 1 patient a thoracic tumour could not be detected with percutaneous US but a diagnosis was obtained by a US guided biopsy of the left adrenal gland (NSCLC).
Inadequate US guided biopsies were performed in supraclavicular regions in 4%(3/74) of lesions and in 3%(1/36) of distant lesions. During a median clinical follow up of 1 year (range 7-41 months) of the 3 supraclavicular non-successfully biopsied lesions, no tumour appeared. The distant non-successfully biopsied lesion was lost to follow-up.

Table 4
Subtyping results of NSCLC in fine-needle aspirations and tissue core biopsies based on morphologic and immunohistochemical analysis

		Fine-needle aspirations	Tissue core biopsies
Local thoracic lesions	Adenocarcinoma	2	0
	Squamous cell ca.	2	1
	Not differentiated	1	1
	subtotal	5	2
Supraclavicular lesions	Adenocarcinoma	30	0
	Squamous cell ca.	11	0
	Large cell carcinoma	1	0
	Not differentiated	1	0
	Not applicable*	2	0
	subtotal	45	0
Distant lesions	Adenocarcinoma	10	2
	Squamous cell ca.	3	0
	Not differentiated	1	0
	subtotal	14	2
	total	64	4

ca, carcinoma
* : 1 patient was known with a pulmonary adenocarcinoma 2 years before and in 1 patient bronchial biopsies already showed a primary adenocarcinoma

Staging of disease
In 58%(61/106) of lesions with a malignant diagnosis, this result confirmed the tumour stage. For local thoracic malignant lesions, supraclavicular malignant lesions and distant malignant lesion these rates were 53%(9/17), 50%(32/64) and 80%(20/25) respectively (table 2).

A total number of 106 lesions with malignancy was found in 102 patients (2 different malignant lesions were biopsied in 4 patients). A pathological confirmation of tumour stage was achieved in 55%(56/102) of patients with malignancy. In 45%(46/102) of patients the biopsy provided a diagnosis but the lesions were not confirmative for disease stage. Ten percent of patients with malignancy (10/102) needed additional invasive investigations to confirm disease stage or subtyping of the tumour. The procedures were lymph node excisions and thoracoscopy by surgery (n=4) and EUS with histologic biopsies for immunohistochemical subtyping of malignant lymphomas (n=1), EBUS for mediastinal node metastasis (n=1) and 4 FNA's (in left adrenal gland, in a coeliac and axillar lymph node, and a cutaneous metastasis, respectively). For the other 36 patients additional biopsies were not required. In 26 patients (25%), PET-CT showed obvious extensive metastatic disease, where confirmation of malignancy seemed not appropriate, 5 patients had mesothelioma, 4 patients had brain metastases, and for 1 patient the biopsy was obtained to differentiate between progression of two different recurrent malignant diseases.

Complications

There were 2 (1.5%) complications registered. Both occurred after tissue core biopsies of a local thoracic lesion. One of the histologic biopsies was complicated by a severe hemoptysis leading to intubation. One of the tissue core biopsies contained also spleen tissue on pathologic examination without adverse events after the biopsy. No other significant adverse events were noticed.

Table 5

Ultrasound guided aspirations and biopsies from PET-CT observed lesions that lead to pathologically confirmed benign diagnoses

	Local thoracic lesions	Supraclavicular nodes	Distant lesions
Non-specific reactive lymph nodes	0	6	7
Organizing pneumonia	2	0	2
Whartin's tumour	0	0	3
Sarcoidosis	0	1	0
Total	2	7	10

DISCUSSION

This study shows that percutaneous US guided biopsies in the diagnosis and staging of thoracic tumours, have a high diagnostic success rate after localization with PET-CT. The quality and quantity of cytologic specimen allowed pathologists to specify subtypes of NSCLC in almost all patients.
The high resolution of US for lesions within about 5 to 10 cm distance from the skin brings many lesions within the scope of US guided biopsies. For intrapulmonary lesions this may not be the case due to positional alterations by gravity when CT images in prone position are used to select lesions for US guided approach in supine position (figure 2). Other failures of US guided punctures were due to inaccurate interpretations of PET-CT like brown fatty tissue located at supraclavicular locations and inaccurate pathologic sampling.

Thoracic lesions

Although US is a relatively underused diagnostic modality, it provides an accurate localisation of chest abnormalities if not surrounded by aerated lung[9]. In this study, the most prominent reason for test failure in local thoracic disease was indeed the inability to image lesions through aerated lung by US. Former studies already demonstrated the benefit of US guided biopsies after CT imaging. Percutaneous biopsies of pulmonary, pleural or mediastinal lesions in contact with the chest wall under US guidance yielded a diagnosis in 90% of patients with a malignancy and in 67% of patients with benign lesions in a study of 124 patients[10]. A high efficacy of US-guided biopsies was reported in 31 out of 34 lesions in the analysis of thoracic tumours[11]. Recently a diagnostic yield of over 93% was reported in a single-session sequential approach of mediastinal masses with US-assisted transthoracal FNA (with rapid on-site evaluation) eventually followed by TCB[12].
Even centrally located tumours with post-obstructive collapse or pneumonitis can be safely biopsied with deep passes and an acceptable diagnostic yield of 74%[13].
Evaluation of local thoracic disease with PET scans has the important advantage to discriminate between active metabolic diseases (inflammatory and malignant) and diseases without increased metabolic activity such as atelectasis, loculated fluid or scar tissue. Lesions without FDG uptake are probably benign and justify a wait and see attitude. Lung cancer is generally characterised by high FDG uptake but for assessment of the T-stage PET scans have limited value[14]. The variety of possible alternative diagnoses of PET-CT positive solitary thoracic tumours account for the need for tissue. This is demonstrated by the high number of benign and non-primarily pulmonary malignancies found in this study (11 out of 19 diagnoses).

Supraclavicular lymph nodes

Supraclavicular nodes are frequently involved in lung cancer and define stage IIIB disease. In this study we focused on PET-CT positive supraclavicular findings and demonstrated malignancy in the majority of patients suspected to have a thoracic neoplasm especially NSCLC. PET-CT has a diagnostic accuracy of 71% in detecting non-palpable supraclavicular lymph node metastasis as was demonstrated in a group

of 32 patients with NSCLC[15]. In our study accuracy was 85%. The sensitivity of palpation of supraclavicular node metastasis in lung cancer is significantly lower than with US or CT. This was illustrated by a study that demonstrated malignancy with US guided FNA in 8% of patients with suspected and potentially operable lung cancer without palpable lymph nodes[16]. A study of 117 patients analysed for lung cancer confirmed this low sensitivity of palpation compared to US or CT and demonstrated that only markedly enlarged metastases were palpable[17].
In patients with proven N2 or N3 disease, enlarged but non-palpable supraclavicular lymph nodes (on CT) contained metastatic disease in even 45.5% of patients[18]. This study confirms the added value of US in detecting enlarged but non-palpable supraclavicular lymph nodes.

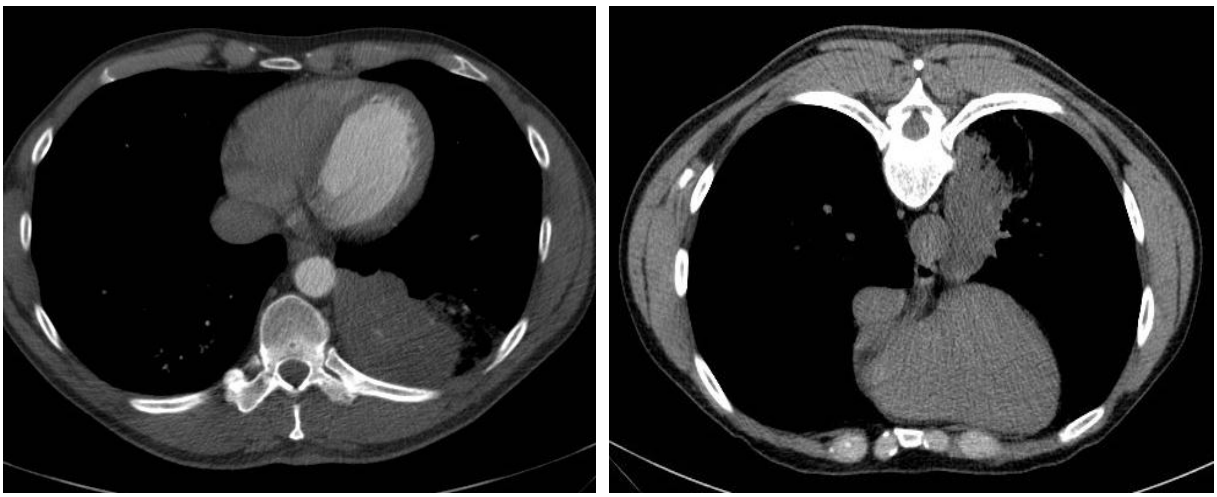

Figure 2 Effect of body position on ultrasound visibility of an intrathoracic lesion. Example of problems encountered with the use of ultrasound.
Left panel, patient in prone position. Lesion in dorsal left lung adjacent to thoracic wall. Right panel, same patient lying in supine position for biopsy. The lesion is no longer adjacent to thoracic wall and becomes invisible for US.

Distant metastatic disease

An important feature of FDG-PET is the ability to detect distant metastatic disease. In about 8-10% of resectable patients with NSCLC and in 18% and 24% of stage II and III patients who were staged conventionally (with CT-scan, bone scans and ultrasonography), PET scan detected distant metastatic disease[19-21].
With US many metastatic sites can be reached percutaneously if lesions are not surrounded by air or covered by bony structures. The ability to visualise lesions is furthermore dependent on size of lesions, tissue characteristics and interfaces, distance to transducer and sensitivity of equipment.

In this study US guided biopsies were performed in axillary, retromandibular, retroauricular, intercostal, soft tissue, skin, and osseous lesions. Half the patients (51%) had non-palpable lesions, the value of US guidance to obtain material for pathologic verification of PET-CT positive lesions is emphasized in this study.

Staging with US guided biopsies.
For more than half of the patients with malignancy in this study, the minimally invasive approach with an US approach confirmed diagnosis and disease stage at one time. Endoscopic investigations, often performed as a routine practice in the analysis of thoracic tumours, are generally considered unpleasant and can be omitted if percutaneous US guided biopsies confirms malignancy.
Diagnostic tests to obtain pathologic specimen should be chosen with a minimal burden to the patient but with maintenance of high quality diagnostic characteristics[5]. US guided biopsies match these qualities as was demonstrated in this study.

Palpation of lesions.
A careful physical examination is one of the first parts of the diagnostic work up of patients with a suspected thoracic malignancy. In this study more than half the lesions were not palpable although the physician was aware of the results of the PET-CT. With the use of US guidance, the lesions could be detected and biopsied very effectively. On the contrary, when lesions are palpable, the use of ultrasound guidance diminishes the chance of inadequate biopsies in thyroid nodules when compared with palpation guided biopsies[22]. Therefore the use of ultrasound is recommended in both situations.

Limitations
Although the study is a prospective analysis of US guided biopsy results, there was no well-defined selection of patients according to protocol criteria. Patients were selected on clinical criteria that work sufficiently in daily practice but influence the outcome of results unequivocally. At the start of a new technique there is a tendency to select lesions with high pretest probability for successfull biopsies. When experience increases, biopsies of more difficult lesions will be attempted but will also lead to increasing failure rates.
A criterion to select lesions for percutaneous US guided biopsies was uptake of FDG. Small lesions, which do not have enough tracer to be seen on PET-CT were not selected for US guided biopsies and could therefore be missed for staging.

CONCLUSION

In conclusion this study demonstrates that US guided percutaneous biopsies increases the pathological outcome in the work up of thoracic tumours after PET-CT. PET-CT in combination with subsequent US guided biopsy provides a definitive diagnosis and tumour stage in the majority of patients and can be performed safely.

Percutaneous US guided biopsies expand the diagnostic capability of pulmonologists in diagnosing and staging thoracic tumours.

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Chapter 6

Analysis of “dry” mesothelioma with ultrasound guided biopsies.

Lung Cancer 2012; 78:229-233

Jos A. Stigt, James E. Boers, Harry J. M. Groen

ABSTRACT

Background: Image-guided sampling of the thickened pleura is a sensitive approach in patients with malignant pleural mesothelioma with pleural effusion. Malignant pleural mesothelioma presenting without effusion however is more of a diagnostic challenge. In this study we report the diagnostic yield and complications of ultrasound-guided cutting needle biopsies in this particular category of patients.
Methods: A retrospective database analysis from September 2007 until January 2012 was performed in 56 patients with malignant pleural mesothelioma. Clinical characteristics and results of diagnostic evaluations were analysed.
Results: Of the 56 patients with malignant pleural mesothelioma, 20 patients presented without pleural effusion of with locular effusion. Ultrasound-guided cutting needle biopsy was performed in 14/20 patients with a diagnostic accuracy of 80%. Only 1 patient had mild hemoptysis immediately following biopsies.
Conclusion: Diagnosing patients with pleural thickenings suspect for malignant mesothelioma without pleural effusion or with loculated pleural effusion is effective and safe with ultrasound-guided cutting needle biopsies.

INTRODUCTION

Malignant pleural mesothelioma MPM is the most common primary pleural malignancy. There is a close relationship with asbestos exposure with a long latency period of up to 40 years before disease develops. The diagnosis is likely in patients with a history of asbestos exposure and clinical symptoms of chest pain, dyspnea and pleural effusion. Imaging supports the diagnosis but pathologic verification on pleural biopsies is almost invariably necessary.
Initially in most patient presenting with a pleural effusion a thoracentesis is performed. Although immunohistochemical staining of embedded cytologic specimen or electron microscopy is sometimes very suggestive for MPM, the obtained tumour cells in pleural aspirations are often too minimal to enable this pathologic elaboration. MPM has a wide range of morphological appearances that makes it difficult to discriminate from other malignancies especially adenocarcinoma. There are no 100% sensitive pathologic markers for MPM and so a panel of markers is used for optimal identification[1]. The acquisition of histologic material is practically obligatory in daily practice to establish the diagnosis of MPM.
Several biopsy techniques have been compared. It was demonstrated that obtaining biopsies under image-guidance provides a higher diagnostic yield than blind biopsies but are still slightly inferior when compared with thoracoscopic biopsies[2].
Abrams needle pleural biopsies (ANPB) performed without image-guidance were inferior compared to CT-guided pleural biopsies[3]. However a comparison of ANPB under CT-guidance (CT-ANPB) with thoracoscopy did not show any difference[4]. For both studies it has to be mentioned that all patients had pleural effusion.
Thoracoscopy has the advantage of immediate evacuation of the pleural effusion during the intervention relieving obviously dyspnea sensations in many patients and enabling adequate pleurodesis at the same time[5].
In a substantial amount of patients however, MPM presents with pleural thickening without effusion. For this category, medical thoracoscopy is hardly possible to carry out and ANPB is impossible. For those cases, image-guided percutaneous approaches with CT or US are appropriate methods to obtain adequate tissue.
There are no direct comparative studies for both modalities in MPM[6]. In a study of 21 patients with MPM the diagnosis was obtained with image-guided cutting needle biopsies (CNB) with CT guidance in 15 patients and with US guidance in 6 patients[7]. In 7/21 patients there was no pleural fluid but unfortunately the study did not specify if the diagnoses were obtained with CT or US guidance.
The largest published series of US-guided biopies in MPM consists of 52 patients. A sensitivity and specificity of 77% respectively 88% was reported. Unfortunately the radiologic appearance with respect to the presence or absence of pleural effusion was not described[8].
In this study we focus on the role of US-guided biopsies as a diagnostic modality especially for MPM presenting without pleural effusion. To that end we characterized all MPM patients by radiologic appearance and assessed retrospectively the way the pathologic diagnosis was obtained in the subset of patients without effusion or with loculated effusion.

PATIENTS AND METHODS

This is a single centre study that analysed the diagnostic procedures in 56 consecutive patients over a period of 4 years with a diagnosis of MPM. For this retrospective analysis there was no ethical approval required. All diagnostic interventions performed to acquire material in the analysis of MPM and all final interventions resulting in the diagnosis of MPM were recorded as well as the radiological appearance of pleural disease.
The results of all US-CNB or attemps to biopt pleural thickenings were recorded as well as gender, age, side of pleural disease, radiologic presentation and histology. Unfortunately, the pleural thickening was not measured with US during the biopsy procedures. The pleural thickness was determined retrospectively by reviewing the contrast-enhanced CT scans and measuring the maximum thickness at the site described in the US guided biopsy reports.
All US-CNB were performed by pulmonologists after informed consent and under local anaesthesia with lidocain 2%. Biopsies were obtained under direct vision with a Hitachi probe (EUP-C532) and a Hitachi EUB-5500 processor. Figure 1 shows an US image of a tissue core needle sampling a mesothelioma and a corresponding CT image.
Bard core tissue biopsy needles 14G x 13 cm. mounted on the US probe with a EZU-PA532 Hitachi Biopsy attachment were used with a Bard magnum biopsy instrument for histologic biopsies. In every patient at least 2 biopsies were taken.

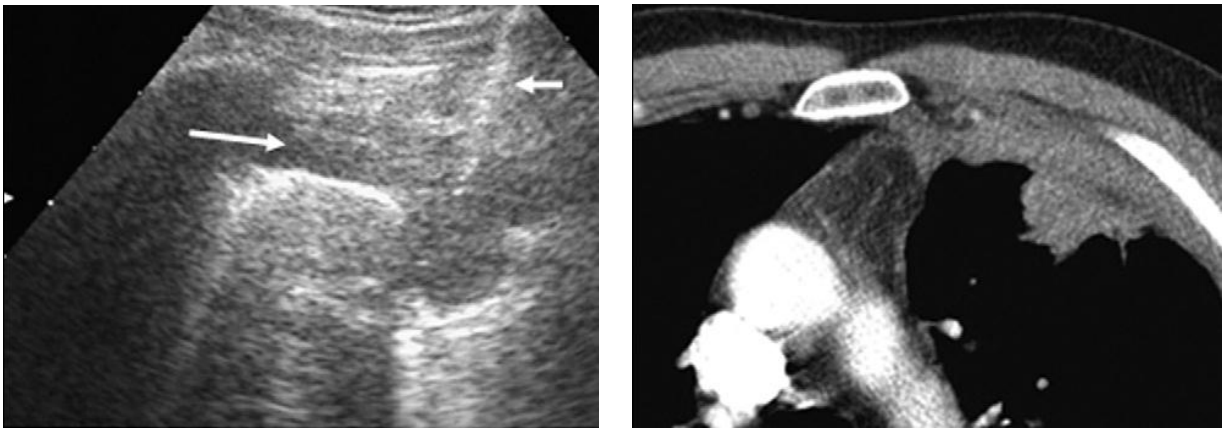
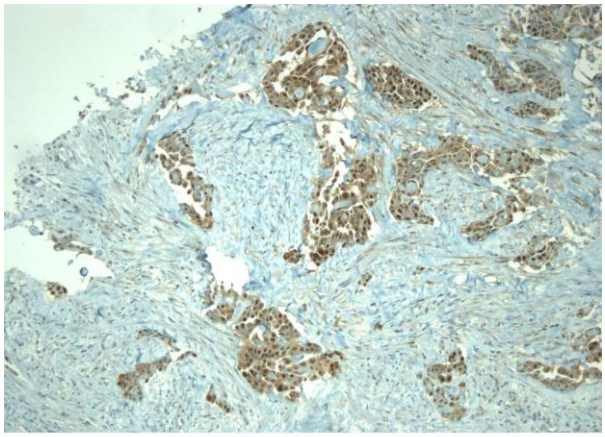


Figure 1. The left panel shows the ultrasound image of a tissue core needle biopsy (14G) from a malignant pleural mesothelioma. The long arrow shows a dark 11 mm thick pleural broadening and the short arrow indicates the needle. Note the local extension of the pleural broadening protruding into the lung. At the left side a rib causes a downward zone of absent ultrasound imaging.
The right panel shows the corresponding CT image.

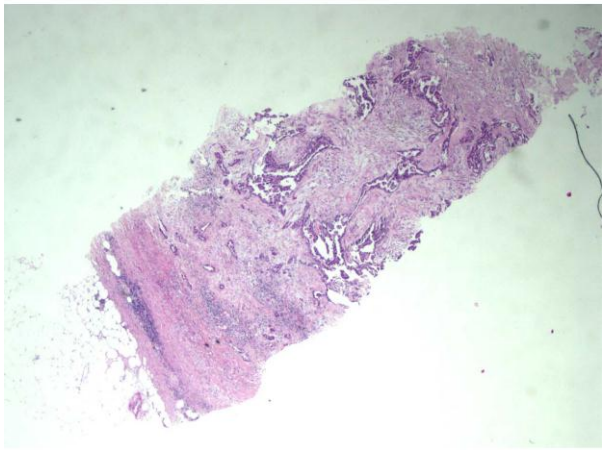
Pathologic examination was performed using Haematoxylin-Eosin stained step sections, in addition with immunohistochemical staining of Cytokeratin AE 1/3, CK5, CK 7, CEA, Calretinin, Ber-EP4, WT-1, CD15, D2-40, TTF-1, MOC-31. Figure 2 shows examples of pathologic slides obtained with 14G needles.
If MPM was certain or suspected, confirmation was requested at the Dutch Mesothelioma Panel (required for damages fee of the Dutch Asbestos Institute).

Figure 2

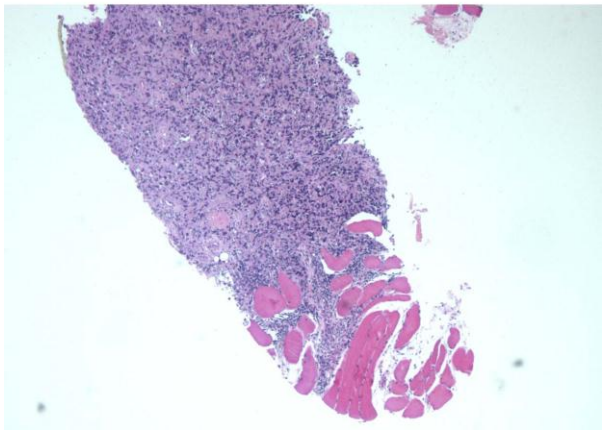
Pathologic slides of tissue core needle biopsies (14G) from malignant pleural mesothelioma.



Panel A: Calretinin immunohistochemistry showing positive nuclei and cytoplasm as brown staining. 40x. Bar: 200 micron



Panel B: Hematoxin and Eosin stained slide showing deaply infiltrative epithelial type mesothelioma. 10x. Bar: 1 mm.



Panel C: Hematoxin and Eosin slide with epithelial type mesothelioma infiltrating thoracic muscles (arrow). 10x. Bar: 1mm.

RESULTS

Diagnostic interventions in mesothelioma

From September 2007 until January 2012 the diagnosis of MPM was made in 56 patients. Table 1 shows the diagnostic interventions that provided the diagnosis and the pathological subtypes.
The patients are classified in 36 patients with a massive pleural effusion and 20 patients with pleural thickenings with or without loculated pleural fluid.
Thoracoscopy provided the diagnosis in patients with massive pleural effusion in 23/36 patients. In 41 patients at least one thoracocentesis was performed but in only 2 patients the results were considered appropriate for the definitive diagnosis. In all other cases, histologic confirmation followed. In only 3/36 patients a surgical diagnostic procedure was performed after negative cytology in aspirated pleural fluid. In 2/3 patients it remains unclear why patients were not analysed with a thoracoscopy and in 1/3 patients there was a hydropneumothorax with lots of adhesions impelling a surgical intervention.
When pleural effusion was not prominent there was a marked predominance of US-CNB (12/20) as provider for the diagnosis of MPM (table 1).

Ultrasound-guided core tissue biopsies in mesothelioma

Table 2 demonstrates the characteristics of 20 out of the total of 54 patients that were analysed with US-CNB. There were 14 patients with only thickened pleura or thickened pleura with loculated effusion who underwent US-CNB and 6 patients with massive pleural effusion. Pleural thickness varied from 7 to 51 mm.
Biopsies resulted in 17/20 in a definitive diagnosis of MPM. In 2 patients a second US-CNB was performed when the first attempts were unsuccessful (table 2).
Non-diagnostic US-CNB was performed in 3/20 patients. In 1 patient (with a circumferential pleural thickening without effusion) the needle could not be forwarded into the pleura although the broadened pleura was visible very well. In 1 patient with massive effusion there was no CT scan available and with US, solid structures for biopsy were not detected. The third patient had US guided cytologic aspiration of a loculated effusion. Pleural thickening could however not be seen and CT scan showed a large MPM mass in a paravertebral location not reachable with CNB.

Ultrasound-guided core tissue biopsies in patients without massive effusions

Table 3 focusses on the subgroup of patients characterised by the absence of massive pleural effusion. In some patients pleural effusion was present but only loculated in a clinical picture dominated by solid pleural thickening.
In 4/20 patients the mesothelioma grew through the intercostal space and was palpable at the outside of the thoracic cage. Upfront diagnostic surgery was performed in 2/4 patients although image-guided biopies should have been easy to perform in both patients afterwards. A palpable chest tube track metastasis was diagnosed with US-CNB in 1 patient after failure of VATS and mediastinoscopy.
The fourth patient with a palpable tumour in the right axilla was diagnosed with upfront US-CNB.

Table 1
Diagnostic tests providing the diagnosis of malignant pleural mesothelioma and results of pathological classification in 56 patients with massive pleural effusion or pleural thickening/loculated pleural effusion.

	Massive exudative pleuritis	Pleural broadening and/or loculated pleuritis	Total
Diagnostic test			
Thoracoscopy	23	0	23
CT-guided biopsies	2	2	4
US-guided biopsies	5	12*	17
Cytologic aspiration	2	0	2
VATS	1	2	3
Minithoracotomy	1	2	3
Abrams biopsy	1	0	1
Surgical biopsy	1	2§	3
Total	36	20	56
Histologic diagnosis			
Epithelioid	27	13	40
Sarcomatoid	5	4	9
Mixed epithelioid/sarcomatoid	4	3	7
Total	36	20	56

CT, computed tomography; US, ultrasound; VATS, video-assisted thoracoscopic surgery
*: including 2 palpable tumours; §: 2 palpable tumours

In 11/20 patients the initially non-diagnostic investigations were performed in the presence of abundant effusion (one or more thoracenteses, ANPB or thoracoscopy). After that the pleural effusion was absent because of chest tube drainage and pleurodesis. These patients proceeded to image-guided CNB with CT (2/11) or US (6/11) or diagnostic surgery (3/11).
Image-guided CNB as initial diagnostic mode was performed in 8/20 patients. Biopsies under CT-guidance in 1/8 and under US-guidance in 7/8.

Complications

A total number of 22 US-CNB was performed in this series. Only 1 patient (the patient with the thinnest circumferential broadening of maximum 7 mm) suffered a single slight hemoptysis. Patients experienced no significant pain and pneumothorax was not observed.

Table 2

Characteristics of patients, radiologic appearance and histology in 20 patients analysed with ultrasound-guided tissue core biopsies

Gender	male: female	19:1
Age	median(range) years	66(57-84)
Side of biopsy	right:left	13:7
Width of biopsied pleura	Median(range) in cm.	17(7-51)
Radiological appearance	Circumferential thickening	5
	Circumferential thickening with loculated effusion	3
	Circumferential thickening and extrathoracic growth	1
	Located pleural thickening	2
	Located pleural thickening with loculated effusion	2
	Located pleural thickening and extrathoracic growth	1
	Massive pleural effusion	6*
Histology	Epithelioid	13
	Sarcomatoid	4
	Mixed epithelioid/sarcomatoid	3
Results US-guided biopsies	Diagnostic CNB	17§
	Non-diagnostic/no biopsies	5‡
Complications	hemoptysis	1

CT, computed tomography; US, ultrasound; CNB, core needle biopsy
*: 1 patient without CT scan.
§: 2 patients had a repeated US-TCB.
‡: non-diagnostic (attempts for) US-TCB followed by repeated US-TNB in 2 patients or by diagnostic surgical procedures in 3 patients.

Table 3
Patient characteristics and biopsy results of all performed diagnostics in 20 patients with a final diagnosis of malignant pleural mesothelioma without massive pleural effusion at time of diagnosis.

gender	male: female	19:1	
age	median(range)	71(56-84)	
side of biopsy	right:left	12:8	
Width of biopsied pleura	median(range) in mm.	17(7-51)	
	Number of patients	Non-diagnostic	Diagnostic
Pleural fluid aspiration*	10	15	0
Abrams biopsy*	3	3	0
Thoracoscopy*	3	3	0
EUS-FNA	2	2	0
CT-TCB	3	2	1
US-TCB	14§	3	12
Surgical biopsy	3	1	2
VATS	3	1	2
(Mini)thoracotomy	2	0	2
Mediastinoscopy	1	1	0

EUS-FNA, endoscopic ultrasound-guided fine needle aspiration; CT-TCB, computed tomography-guided tissue core biopsy; US-TCB, ultrasound-guided tissue core biopsy; VATS, video-assisted thoracoscopic surgery
*: performed in loculated effusion or initially in massive pleural effusion before pleurodesis
§: in 1 patient 1 non-diagnostic US-TCB was followed by a second diagnostic US-TCB

DISCUSSION

Diagnosing malignant pleural mesothelioma is often a challenge in daily practice especially when the disease presents with diffuse or localised pleural thickening rather than pleural effusion.
Pleural thickening without effusion is sometimes the first clinical presentation or remains as the final situation after former diagnostic interventions with relieving chest tube drainages. This study describes the experience with US-CNB in this particular subset of patients with MPM.
A diagnostic accuracy of 80% was demonstrated for US-CNB in patients with MPM. In only 2/14 patients, US-CNB was non-diagnostic (although in 1/14 patients US-CNB had to be repeated). These 2 patients were diagnosed with VATS and minithoracotomy. A diagnostic accuracy for US-CNB of also 80% was described in a study of 70 patients suspected for MPM[8]. This study is however incomparable to our report because it did not focus on the subgroup of patients without massive

effusion. In a study of 33 patients with a suspected malignant effusion, in 21 patients a definitive diagnosis of MPM was obtained with image-guided CNB. A diagnostic accuracy of 86% was reported but concerned CT-CNB as well as US-CNB[9]. This result is neither comparable with ours because two thirds of patients had pleural effusions and only 6/21 had US-CNB[10].
In retrospection, 2 out of 4 upfront surgical diagnostic procedures in our series could have been precluded when US-CNB was tried before. Both patients had MPM locations palpable on the outside of the thorax and were analysed before imaging. For the 2 other patients US-CNB was less suitable. In 1 patient a non-diagnostic CT-CNB was performed and 1 patient had a pleural thickening of maximum 8 mm. considered too small by the treating physician for image-guided biopsies.
In 2/20 patients a successful CT-CNB was the chosen diagnostic investigation but for both the diffuse pleural thickenings could have been approached with US-CNB as well.
The advantages of US guidance over CT-guidance is its ready availability and lack of radiation exposure but above all the possibility of real-time sampling which reduces the chance of lung perforation and perforation of larger blood vessels. The direction of the needle and the depth of the biopsy can be established very well with the help of US. In 2 patients diagnosed with US-CNB the MPM was palpable at the outside of the thoracic cage. Although US-guidance is less essential in this situation, it still assists the clinician by determining the needle track and visualizing surrounding structures.
US-CNB was also performed in 6 patients with a massive pleural effusion. All but one of these US-CNB were successful and the failure was repeated successful under US-guidance at another moment. Although very feasible and patient-friendly, CNB is only diagnostic and does not treat patients as does thoracoscopy. So for patients with massive pleural effusion thoracoscopy is the diagnostic procedure of choice.
The technique of US-CNB is safe. In this study only 1 patient had mild hemoptysis. This corresponds to the small numbers of complications reported in literature. Studies in 53, 6 and 5 patients with MPM (diagnosed with US-CNB) reported complications in 2, 1 respectively 0 patients[8, 10, 11]. The 3 patients had mild hemoptysis, local chest pain and a chest wall hematoma.
The problem of needle track seeding was studied in 100 patients with MPM who were diagnosed with image-guided CNB or surgical biopsies (thoracoscopy or thoracotomy). In 22/100 patients CNB was performed (1/22 patients with US-guidance) and only 1 patient developed a needle track seeding. This contrasted to the 22% of needle track seedings that were found in patients diagnosed with surgical procedures[12]. Unfortunately, we did not monitor for this disease manifestation in our study. In fact 2 of our patients were diagnosed primary on needle track metastases; 1 after former surgical procedures and 1 patient a year after a negative thoracocentesis.
Limitations of this study are its retrospective nature and its small number of patients. The limited number of CT-guided biopsies do not allow a comparison between US and CT as guiding modality. Nevertheless this particular category of so called “dry” MPM patients and the diagnostic capacity and suitability of US-CNB for these

patients deserves attention. US-CNB can eventually prevent more aggravating surgical procedures.
In conclusion, this study demonstrates the safety and high diagnostic accuracy of US-CNB for patients with MPM presenting without massive pleural effusion. Percutaneous biopsies and aspirations with US for indications such as pleural fluid or pleural pockets are broadly applied by pulmonologists nowadays so it seems a logical and small step to expand the use of this technique to solid pleural thickenings as are seen in MPM.

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Chapter 7

Mediastinal Incidentalomas

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Jos A. Stigt, James E. Boers, Ad H Oostdijk, Jan-Willem K van den Berg, Harry J.M. Groen,

ABSTRACT

Introduction: Incidental mediastinal lymphadenopathy challenges pulmonologists to decide on eventual further diagnostic steps. The aim of this study was to characterize unexpected mediastinal findings by imaging and pathologic analysis.
Methods: Entry criterion for this prospective explorative study was mediastinal lymphadenopathy as an incidental finding on CT scans made for indications other than the analysis and staging of neoplasms.
Lymph node dimensions were measured on CT. Subsequent diagnostic investigations were positron-emission tomography (PET), endoscopic ultrasound (EUS) or endobronchial ultrasound (EBUS) guided punctures and clinical follow up.
Results: 83 patients from 8 hospitals met the entry criteria. The median number of Naruke stations with enlarged nodes was 7 (range 3-9). The median size of all nodes measured, varied between 6 and 14 mm. The median number of lymph node stations with nodes of at least 10 mm was 3 (range 0-8).
Hilar node enlargement was detected in 77% of patients.
No definitive diagnosis was obtained in 7/83 (8%) of patients. Lymphocytes were found in 55/83 (66%) and sarcoidosis in 18/83 (22%) of aspirates.
PET showed metabolic activity in 87% of patients.
Follow up CT scans were available in 36/62 (58%) of patients without a classifying diagnosis. Two patients developed lung cancer 2 years after initial analysis.
Conclusion: Incidental mediastinal lymph nodes on CT are characterized by multiplicity, relative small sizes and coexistence with hilar lymphadenopathy in the majority of patients. These nodes often display increased metabolic activity.
The low predictive value for malignancy justifies a restrictive attitude towards invasive diagnostic testing.

INTRODUCTION

Enlarged mediastinal lymph nodes may be detected incidentally on computer tomography (CT) of the chest and often raise the question whether further analysis is indicated. Nowadays CT is easily accessible and made for a broader range of indications including pulmonary embolism and coronary artery imaging. Thus, incidentally enlarged mediastinal nodes will be found in incremental numbers challenging the pulmonologist to make decisions about further diagnostic approach. Incidental findings are defined as imaging abnormalities not related to the indication for which the CT is requested.
Prevalences of incidental mediastinal lymphadenopathy are reported between 0.15% and 3%, but a systematic pathological analysis of these findings has not been described so far[1-9]
Several studies describe pathologic results of mediastinal masses in patients of unknown etiology[10-13] The rates of malignancy ranged in these studies from 29% to 65% with entry criteria that varied significantly. A meta-analysis showed that larger nodes have a higher chance of containing malignancy[14]. The prevalence of malignancy will be lower when nodes are small as is usually the case when detected by incidence on CT.
This prospective study with patients referred from eight different hospitals describes the extend, size, metabolic activity and pathological findings of incidentally detected mediastinal lymph nodes.

PATIENTS AND METHODS

Study entry criterium

Patients with at least one enlarged mediastinal lymph node (shortest diameter of ≥ 10 mm) were selected for the study when this incidental finding was detected on CT of the thorax made for a wide range of indications other than the analysis or staging of any neoplasm. One patient was analysed although the shortest diameter of nodes did not reach 10 mm. Excluded were patients with a poor performance score precluding the consequences of further diagnostic analysis.

Imaging and evaluation of nodal disease

Patients were referred from eight different hospitals. CT of the thorax was performed according to local protocols. One of the authors (JS) reviewed all CT scans and all visible mediastinal nodes were measured along the shortest axis and the mediastinal position was classified according to the International Association for the Study of Lung Cancer (IASLC)[15].
In only a limited number of patients a positron-emission tomography (PET) was performed with fluoro-2-deoxyglucose (FDG) in the mediastinal nodes was determined (GE Discovery ST PET-CT scanner; General Electric, Milwaukee, Wis).

Ultrasound guided biopsies and pathologic analysis
Mediastinal lymph nodes were approached with EUS or EBUS for a diagnostic aspiration or biopsy. EUS and EBUS was performed with Pentax ultrasound endoscopes with a Hitachi EUB-5500 processor. The fine needle aspiration (FNA) biopsies were performed under conscious sedation with midazolam and with local anaesthesia that was sprayed in the oropharynx (lidocain 1%) and lidocain gel 20 mg/ml. Per nodal site, three to four needle passes were performed and at least two aspirates were smeared on slides. Remaining aspirate was deposited in a fixative medium (Carbowax 2% [polyethyleenglycol 20 g in ethanol 96% methylated and filled up to 1000 ml with water]) for immunohistochemical staining.

RESULTS

Patient selection
From August 2005 until December 2010, 83 out of 1530 patients met the inclusion criteria of our study. They underwent EUS or EBUS for analysis of incidentally detected mediastinal lymphadenopathy and if possible FDG-PET. CT was always available.

Patient characteristics
Table 1 shows the characteristics of 83 patients (61 men and 22 women) with a median age of 59 years (range 27-87).
All patients were referred for EUS-FNA (81) or EBUS-TBNA (2) of incidentally detected lymph nodes on CT scans for a variety of indications (table 1). Most CT scans were angio-CT scans made in the work up of pulmonary embolism (43). Other indications were pulmonary infiltrative disease (5), pleural disease (8), parenchymatous disease (3), health screening scans (2), coronary angiography (2) and a remaining group with other indications (20).

Numbers and sizes of mediastinal nodes
In our patients nodes of all sizes were visible on CT-scans in at least 3 levels (according to the Naruke classification) and in some patients lymph nodes were visible on CT at all sites. The median number of sites with visible nodes was 7 (range 3-9). The median number of lymph nodes of at least 10 mm was 3 (range 0-8).
Table 1 shows the numbers of patients with different lymph node sizes at any mediastinal site. The median number of involved mediastinal sites is also described in table 1. In 64 patients hilar lymphadenopathy was also evident. In 19 patients hilar node enlargement was absent.
Table 2 demonstrates the nodal size, subdivided in several size categories, for specified mediastinal sites.

FDG uptake in mediastinal nodes
PET scans had been performed in 29 patients. Increased FDG-uptake in mediastinal nodes was demonstrated in 25 patients (3 inadequate aspirates, 16 reactive lymphoid change, 5 granulomatous inflammation compatible with sarcoidosis and 1

Tabel 1
Patient characteristics of 83 incidentally encountered mediastinal lymphadenopathy

	N	Median (Range)
Patients	83	
Age (yr)		59 (27-87)
Gender		
Male	61	
Female	22	
Indications for CT scan		
Suspected pulmonary embolism	43	
Pleural disease on chest X-ray	8	
Suspected interstitial pulmonary disease	8	
Persistent pulmonary infiltrate on chest X-ray	5	
General Internal analysis	5	
General Pulmonologic analysis	3	
Imaging of benign pulmonary abnormalities	3	
Commercial screening	2	
Coronary angiography	2	
Analysis ascending aorta	2	
Other*	2	
Mediastinal locations		
All measurable nodes	83	7 (3-9)
Nodes of at least 10 mm.	82	3 (0-8)
10-15 mm	79	3 (1-6)
16-20 mm	38	1 (1-4)
21-30 mm	21	1 (1-3)
> 30 mm	2	1
Hilar nodal enlargement		
Yes	64	
No	19	

*: mediastinal lymphadenopathy as incidental finding on MRI for cervical radicular syndrome and exclusion of a primary malignancy in the workup of an abnormal cerebral MRI .
MRI, magnetic resonance imaging; CT, computed tomography.

granulomatous inflammation staining positive for mycobacteria) In four patients there was no increased FDG uptake observed (all reactive lymphoid change).

Pathologic analysis

Table 2 describes the results of pathological analysis. Cytologic aspirates were derived from 80 patients. In two patients introduction of the endoscope failed and in one patient, nodes were not detected with ultrasound. In four patients the aspirates were considered inadequate for pathologic analysis.

Guided by ultrasound, subcarinal nodes were biopsied in 67 patients, the aortic window (locations 4L and 5) 34 patients, location 8 in two patients and locations 2L and 4R both in one patient. In 76/80 patients the quality of aspirated material allowed an adequate pathological analysis.

In 55 patients the aspirates showed many lymphocytes and were considered reactive without a classifying diagnosis. Eighteen patients had a granulomatous inflammatory reaction compatible with sarcoidosis. One patient also had a granulomatous disorder and acid-fast mycobacteria with Ziehl-Neelson stain. Conventional culture and a polymerase chain reaction for Mycobacteria were negative. One patient had a bronchogenic cyst.

In a patient with a history of breast cancer, a CT was made to analyse loculated pleural fluid without pathological evidence of a malignancy. The enlarged mediastinal nodes, not visible on normal X-ray, contained metastatic disease of her breast cancer.

In a subset of 21 patients with conditions frequently associated with mediastinal lymphadenopathy (pleural disease, interstitial disease and infiltrative disease) lymphocytes were present in 81%, and a granulomatous inflammatory pattern in 14% compared to 61% and 24% for all other patients, respectively.

Follow up

A classifying diagnosis was obtained with EUS in 21 out of 83 patients. In the 62 patients with no classifying diagnosis, follow up data were analysed. This group consisted of 55 patients with adequate aspirates, 4 patients with inadequate aspirates, 2 patients in whom introduction of the echoscope failed and 1 patient in whom no enlarged lymph nodes were detected with ultrasound.

Follow up CT scans were available for 36/62 patients (58%). The scans were made after a median interval of 118 days (range 10-692 days). Decisions for follow up were made at the discretion of patients own treating physician. Eleven patients were referred from other hospitals and lost to follow up and for 16 patients in our own practice, no follow up CT scans were requested.

In 9 patients the lymphadenopathy had reduced spontaneously. In 24 patients the lymphadenopathy remained unchanged and in 2 patients the lymphadenopathy progressed both in size and in number. In both patients with progressive lymphadenopathy detected on CT scans after 20 and 23 months of follow up, the initial EUS-FNA contained adequate samples without malignant cells.In one patient, a repeat EUS-FNA after 21 months in subcarinal lymph nodes showed malignant cells and this patient was diagnosed with stage IV NSCLC. In the other patient,

metastatic lung cancer was demonstrated by a liver biopsy 22 months after initial EUS-FNA. In this patient with obvious progression of mediastinal lymphadenopathy, metastatic disease was not verified as a repeated EUS was not performed. Revision and comparison of CT scans suggests progression of already existing lymphadenopathy but an evident primary tumour was absent on the initial scans. When the number of enlarged nodes and the sizes of enlarged nodes for the patients with malignancy (developing during follow up or at initial analysis) are compared with patients without malignancy, there are no differences.

Table 2
Nodal distribution and pathological results of EUS and EBUS

	Radiologic distribution of mediastinal nodes (according to IASLC)					**Size of Nodes (mm) Median (Range)**	
	< 10 mm	**10-15 mm**	**16-20 mm**	**21-30 mm**	**>30 mm**		
2R	41	18	9	1	0	8	(4-26)
4R	26	37	15	4	0	13	(4-27)
2L	39	9	0	0	0	6	(3-15)
4L	35	34	2	0	0	10	(4-16)
5	25	37	8	0	0	11	(5-19)
6	46	18	4	0	0	8	(3-20)
7	8	35	16	18	2	14	(6-35)
8	10	18	6	1	0	11	(4-28)
9	14	7	0	0	0	8	(5-15)

Pathologic results

Lymphocytes	55
Sarcoidosis	18
Metastasis mammacarcinoma	1
Granulomatous inflammation, ZN positive stains	1
Bronchogenic cyste	1
Inadequate needle aspirate	4
No aspirate acquired	3

EUS, endoscopic ultrasound; EBUS, endobronchial ultrasound;IASLC, International Association for the Study of Lung Cancer; L,left; R,right; ZN, Ziehl-Neelsen

DISCUSSION

Imaging

In this study a group of 83 patients referred for suspected pulmonary malignancy were described who had in common that mediastinal lymphadenopathy was detected incidentally on CT performed for a variety of indications other than the analysis of malignancy.

The most characteristic feature of these enlarged mediastinal lymph nodes is their presence in multiple Naruke stations, its variable but relatively small size and the concomitant hilar involvement in most patients. There was one patient in whom the largest nodes measured less than 10 mm. In 46% (38/83) patients the largest measured nodes were between 10 and 15 mm followed by 26% (22/83) patients with maximum nodes of 16-20 mm, 24% (20/83) patients with largest nodes of 21-30 mm and 2% (2/83) with nodes measuring more than 30 mm.

The size of mediastinal nodes is predictive for the final pathologic diagnosis as has been demonstrated in a study where benign nodes were significantly smaller than malignant nodes[16]. However the nodes of the patient in this study with breast cancer metastasis and the two patients that developed NSCLC during follow up did not differ in size from the other patients.

Incidental mediastinal findings are described in screening studies for coronary artery disease but information on extend and size of these findings is scarse[1, 2, 4-7]. Lung cancer screening studies also report prevalences of nonpulmonary incidental findings but details of mediastinal incidental findings are limited or absent[3, 8, 9].

If imaging was expanded with FDG-PET, the majority of patients demonstrated increased FDG uptake in their nodes.

It is debatable whether PET is indicated in patients without radiologic evidence of primary lung tumours. All but one of 29 patients in whom PET was performed had at least 1 lymph node measuring 10 mm or more in short-axis diameter. Remarkably, the only patient with nodes smaller than 10 mm developed lung cancer with confirmed subcarinal metastatic disease.

PET offered in our study no added value to CT in incidentally detected mediastinal nodes since reactive inflammatory diseases also showed metabolic activity and hence did not discriminate from malignant disease.

Pathology

In our study, the majority of incidental mediastinal findings showed a reactive inflammatory pattern on pathological analysis sometimes compatible with sarcoidosis as might be expected from imaging results. Granulomatous inflammatory reactions could be expected relatively more often in patients with conditions with pleural, interstitial or infiltrative disease.In a screening study in asbestos workers in 1% of patients mediastinal lymphadenopathy was reported and all lesions were proved to be benign[9]. The size of the detected mediastinal nodes was not provided but since this was a screening study, the lesions were presumably small in size.

In a study of 1520 lung cancer screening participants, incidental mediastinal findings (2 lymphomas) were reported but the prevalence of incidentally detected mediastinal lymphadenopathy was not described[8]
Malignancy was found in only one patient in our study. This patient had a history of breast cancer and so this finding was not unexpected. In all other patients, malignancy was not observed. These findings conflict with the high pretest probability of malignancy in patients with mediastinal lymphadenopathy of unknown aetiology in former series[10-13]. Critical is how patients are selected. In a study of isolated mediastinal lymphadenopathy, EBUS-TBNA resulted in a diagnosis of malignancy in 60% (33/55) patients[12]. All patients in this study had suspected lymphoma, 29% (16/55) patients had a history of malignancy and excluded were patients with typical clinicoradiologic features of sarcoidosis. This study did not specify the size and number of mediastinal nodes. Neither were these features described in two studies reporting malignancy in 29% (40/140) and 65% (22/34) of patients[10, 13]. Finally, in 61% (46/75) of patients malignancy was observed in subcarinal nodes but these were at least 25 mm[11]. In a subgroup of patients analysed for isolated mediastinal lymph nodes with typical clinicoradiological features suggestive for sarcoidosis this diagnosis was confirmed in 93% (26/28) patients but 1/28 had malignancy[17].
Our study population cannot be compared with the patients in these studies due to different entry criteria and lacking information on number and size of mediastinal nodes.
Regarding our own results in patients with high rates of hilar lymphadenopathy and multiple mediastinal nodes the number of patients with a granulomatous inflammatory reaction seems small. The difference of these results with our findings can be explained by the fact that the incidental nodes in our study were relatively small in size and mostly could not be detected with normal chest radiographs.

Follow up

In the literature there are no follow up data of incidental mediastinal findings. In our study with a deliberate follow up, determined by the treating physicians, follow up CT was available in more than half the patients without a classifying diagnosis of incidental mediastinal findings. Two out of 62 patients (3%) developed lung cancer in nodes that had been evaluated initially with EUS-FNA. The interval of detection of lung cancer was almost 2 years in both patients. Based on follow up findings in this study, the need for regular monitoring of incidentally detected lymphadenopathy with CT scans is debatable.
Regarding the pathologic diagnoses in this study, the probability to detect a disease that needs treatment is very low (although an exception could be made for patients with a history of malignancy). This counterweights the low threshold to apply modern endosonographic techniques due to their favorable safety profile and high diagnostic accuracy[18, 19].

CONCLUSION

Incidentally detected lymphadenopathy often worries pulmonologists leading to further diagnostic procedures. This explorative study demonstrates that incidentally detected mediastinal findings are mainly a manifestation of reactive inflammatory origin in mainly multiple, slightly enlarged mediastinal lymph nodes. Our study does not support a very aggressive diagnostic approach of these nodes except for patients with known malignancy or a history of malignancy.

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Chapter 8

A diagnostic program for Patients Suspected of Having Lung Cancer

Clin Lung Cancer 2012;13:475-81.

Jos A. Stigt, Steven M Uil, Ad H. Oostdijk, James E. Boers, Jan-Willem K van den Berg, Harry J. M. Groen

ABSTRACT

Background: A standardized diagnostic program, initiated to reduce the length of the diagnostic track and to improve application of diagnostic tools for patients referred with suspicious abnormalities on standard chest radiographies, was evaluated.
Methods: The findings on integrated PET-CT determined the choice of invasive investigations to be performed the same day. Diagnostic results, time courses and number and sorts of applied invasive investigations were assessed.
Results: In 297 eligible patients, malignant disease was diagnosed in 72% and benign disease in 26% of patients. One percent of the patients had no abnormalities at all.
For 85% of patients with a malignancy, investigations were rounded off in one day resulting in a diagnosis and definitive clinical disease stage.
The median time from start of the analysis to informing the patient about diagnosis and tumour stage was 7 days.
One invasive investigation was performed in 53% of patients in the study group and at least 2 investigations were performed in 33%. Bronchoscopies formed a part of the diagnostic process in 59% of patients. Surgical diagnostic procedures were performed in 8% of patients.
Conclusion: The diagnostic program resulted in a short time to diagnosis with finalization of invasive investigations on one day in the majority of patients. The imaging-based choice of invasive investigations precluded bronchoscopies in a substantial part of the patients.

INTRODUCTION

Patients have to undergo a variety of diagnostic tests for the analysis of suspicious abnormalities on standard chest radiographies to obtain information on pathology and extend of disease. In case of malignancy, tumour stage, resectability, operability and comorbidity are important issues to assess for optimal patient management.
A short diagnostic track may lead to more satisfaction for patients and physicians although a relationship between hospital waiting time and survival or recurrence rates in lung cancer patients has never been demonstrated[1-7]. Diagnostic time is considered as an indicator for quality and recommendations on this issue are published in guidelines.
Former publications on fast-track diagnostic programs used bronchoscopy after positron-emission tomography (PET) and /or computerized tomography (CT) as standardized work-up[8, 9]. The diagnostic accuracy of bronchoscopy for mediastinal staging however is limited and distant metastatic disease still needs verification. So for many patients in these programs, further diagnostics are required.
Our diagnostic program was designed to perform all tests, needed to diagnose suspicious abnormalities, on one single day (including confirmatory tests for disease stage in case of malignancy). PET-CT, as superior imaging modality for lung cancer suspects, guided the choice of further investigations[10-12]. Relevant findings on PET-CT were confirmed by bronchoscopy, ultrasound (US) guided punctions, endoscopic ultrasound with fine-needle aspiration (EUS-FNA) or endobronchial ultrasound with transbronchial needle aspiration (EBUS-TBNA) or combinations.
We hypothesized that the sequence of investigations and the choice from a variety of immediate available biopsy techniques should lead to a high rate of finalized diagnostic work-ups at one diagnostic day. We expected that this would result in a short time to analyze patients with suspected lung cancer. We also expected that many patients would be diagnosed with ultrasound guided biopsies (either endoscopic or percutaneous) that would be sufficient for a proper diagnosis including tumour stage. This would demonstrate consequently that a diagnosis of thoracic malignancy not automatically requires a bronchoscopy.

PATIENTS AND METHODS

Study population

The target population consisted of consecutive patients with abnormalities suspicious for lung cancer on standard chest radiographies referred to the outpatient clinic of the Chest Unit by a general practitioner or medical specialist from February 2006 until January 2010.
After the radiographs and referral notes were reviewed by the author (JS), patients were invited to enter the study after answering a questionnaire by telephone. The questionnaire was used to inform and invite patients to participate in the program and to obtain information on basic patient characteristics, demographics, comorbidity and medication use. The study was approved by the Medical Ethical Committee of the Isala Clinics in Zwolle, the Netherlands and informed consent was obtained from all

patients. Patient information forms and a time table for the diagnostic day were provided by mail or email.
A cost analysis was not performed.

Diagnostic workup
The program was designed to enable the analysis of three patients on a fixed day once a week (figure 1).

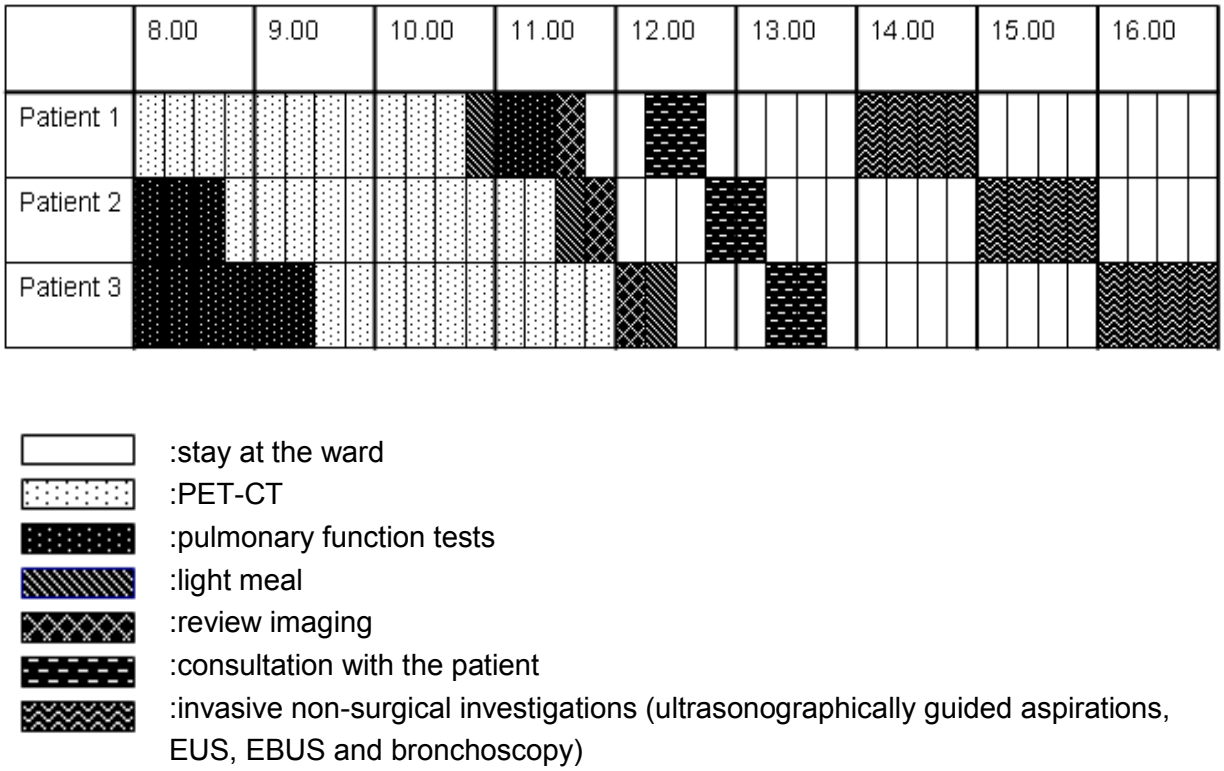

Figure 1. Time table of activities and investigations for 3 patients (maximum) during the day of the diagnostic program.

PET-CT, positron emission tomography/computed tomography; EUS, endoscopic ultrasonography; EBUS, endobronchial ultrasonography

During the diagnostic day in the clinic the patients were accompanied by a nurse. Routine laboratory tests were performed before PET-CT scanning. EKG and pulmonary function tests were performed in the morning hours. Every week, 3 PET-CT slots were reserved for the program. The slots were reallocated 48 hours before the time slot to the general hospital when less than 3 patients entered the study in that week. PET-CT was performed on an integrated PET-CT scanner (GE Discovery ST PET-CT scanner; General Electric, Milwaukee, Wis) and reviewed by the author

(JS) and a nuclear physician (AO). All CT scans were contrast CT scans except for patients with renal insufficiency. Afterwards patients consulted a pulmonologist for history, short physical examination, explanation of test results so far and forthcoming invasive investigations in the afternoon. The choice of these invasive non-surgical investigations was guided by a flow-chart (figure 2) and consisted of bronchoscopy (eventually with blind TBNA), US guided FNA (US-FNA) or US guided core tissue biopsies and EUS. Navigational bronchoscopy for peripheral lesions was not performed in this study. EBUS became available halfway the study. The invasive diagnostic test to be chosen, had to provide ideally a diagnosis and tumour stage at one time.

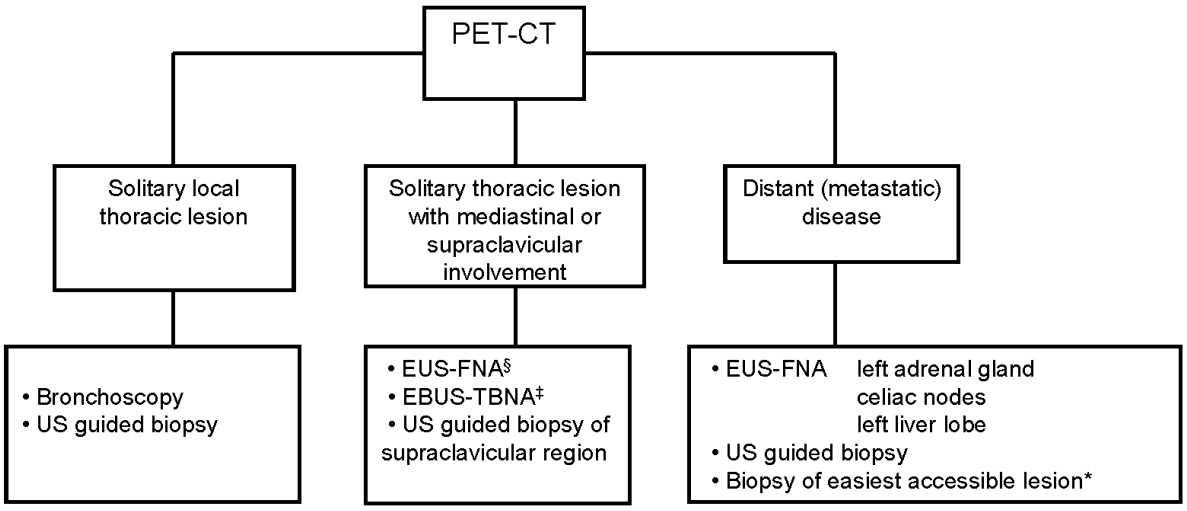

Figure 2. Diagnostic flow chart.

§ :lymph nodes on Naruke 2L, 4L, 5, 7, 8 and 9, very large nodes on 2R and 4R

‡ :lymph nodes on Naruke 2L, 4L, 2R, 4R, 7 and hilar masses

* :when PET-CT shows multiple pulmonary, osseous or hepatogenic lesions that are not easy accessible and demonstrate obvious metastatic disease

PET-CT, positron-emission tomography; US, ultrasound; EUS-FNA, endoscopic ultrasound-guided fine needle aspiration; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration

Histologic and cytologic specimen were analysed on a routine base. The time for pathologic analysis varied between one day in case of high clinical priority to maximally five working days. In cases with only cytologic specimen, NSCLC subtype determination was performed by using immunohistochemical stained slices of paraffin embedded cell suspensions. Molecular analysis was routinely implemented halfway 2009 in patients with stage IV adenocarcinoma.
At the end of the diagnostic day, the patients left the hospital with an appointment for a final consultation one week later to be informed about the diagnosis and treatment.

If additional investigations were needed (particularly exercise tests, perfusion scans or preoperative evaluations by other consultants), appointments were made. Brain MRI was not part of the diagnostic process so this was performed on separate days (within a week) in patients with clinical stage III disease or clinical suspicion of brain metastasis.

Evaluation of results

Numbers of patients with benign and malignant disease and patients in whom PET-CT revealed no abnormalities at all, was evaluated. Malignant and benign disease was subdivided in clinical diagnoses and pathologically confirmed diagnoses.
The percentage of patients with a definitive diagnosis (including disease stage) obtained during the diagnostic day was determined. Version 7 of the International Association for the Study of Lung Cancer (IASLC) was used for staging of NSCLC[13].
The number of days (including weekend-days), from initial abnormal chest radiography to first visit at the pulmonary department was assessed as well as the time from start of analysis to the final consult (the moment patients were informed about the definitive diagnosis including disease stage and proposed therapy) and the time from final consult to start of treatment.
The numbers of invasive tests (bronchoscopy, EUS-FNA, EBUS-TBNA and percutaneous US-FNA) and surgical diagnostic procedures performed were determined.

RESULTS

Diagnoses

A total of 303 patients were enrolled in the program from February 1st 2006 until January 1st 2010. Six patients were ineligible for analysis (4 patients already had CT-scans performed before the study day, one patient had no PET scan at the planned diagnostic day and one patient underwent a supraclavicular biopsy before inclusion). Of 297 patients included, 72%(215/297) had malignant disease (202/215 patients with a pathologically confirmed diagnosis and 13/215 patients with a clinical diagnosis). Benign disease was diagnosed in 26%(78/297) patients (9/78 patients with a pathologic diagnosis and 69/78 patients with a diagnosis based on imaging). No abnormalities at all were detected on PET-CT scans in 1%(4/297) of patients. In these patients, supposed lesions on chest radiography appeared to be superposed normal structures (table 1).

Benign diagnoses

Table 2 shows characteristics for 78 patients with a benign diagnosis with a preponderance of infectious diseases (52%). Metabolic activity was seen in most patients with an infectious etiology and in all patients with sarcoidosis and pulmonary embolism. The diagnosis was based on pathology in 12%(9/78) and on imaging in 88%(69/78) of patients.

A median time of 9 days passed from the day the abnormal radiograph was made until the patient entered the diagnostic program (range 2-33 days). Most patients were informed about their benign diagnosis immediately at their first contact with the pulmonologist when conclusions were based on imaging with PET-CT. Treatments with antibiotics (11), anticoagulation (3) and tube thoracostomy (2) were initiated the same day. One patient was scheduled for rigid bronchoscopy on a separate day. Nine patients had to wait for the results of pathologic analysis.

Table 1
Results of the diagnostic program

diagnosis	No of patients (%)
Malignant Disease¶	
Pathological diagnosis	202 (68)
Diagnosis based on imaging	13 (4)
Benign Disease§	
Pathological diagnosis	9 (3)
Diagnosis based on imaging	69 (23)
No abnormalities diagnosed	4 (1)
Total	297

¶ : subdivided in table 3

§ : subdivided in table 2

For the patients with a diagnosis based on imaging (69/78), radiological follow up data were collected in 71%(49/69) of patients (11 with CT and 38 with chest radiographs). During a median follow up of 117 days (range 13-1146 days), one patient developed a pleural mesothelioma and in one patient a non-suspected lesion was resected and turned out to be a NSCLC. There was no radiological follow up in 29%(20/78) of patients because the diagnosis was considered clear-cut.

Malignant diagnoses

Characteristics, diagnosis and tumour stage of 215 patients with a diagnosis of malignancy are described in table 3. NSCLC was diagnosed in 74%(159/215) of patients, SCLC, metastases from non-pulmonary origin and primary non-epithelial malignancies in 13%(29/215), 6%(13/215) respectively 6%(12/215) of patients.
For almost all patients, a pathologic subtyping of NSCLC was available as shown in table 4.

Table 2
Benign diagnoses in study group

	No of patients	**(%)**
Age (range,y)	63 (36-81)	
Sex	49	63
male	29	37
female		
Diagnosis		
Infectious Diseases[§]	42	54
Benign tumours	9	12
Scar tissue	4	5
Sarcoidosis	5	6
Vascular structures	3	4
Pulmonary embolism	3	4
Pleural thickenings	2	3
Rounded atelectasis	2	3
Miscelaneous[¶]	8	10
Total	78	

§ : 40 recovering pneumonias; 1 lung abcess; 1 atypical mycobacteriosis
¶ : 1 mediastinal lymphadenopathy without FDG uptake on PET scan; 1 thyroid; 1 atelectasis; 1 reactive pleural fluid; 1 paralytic diaphragm; 1 solitary pulmonary nodule with ground glass appearance and positive on PET scan; 1 ostheophyte; 1 sequestrated segment

In 5%(11/215) of patients the diagnosis of NSCLC was based on imaging but pathological confirmation was not obtained. Eight of these patients had early disease and were offered curative radiotherapy and 3 patients (high age and bad general condition) had advanced disease but further analysis was abandoned because of lack of clinical consequences.
In 15%(32/215) of patients the diagnostic day did not provide enough information for a final diagnosis (including tumour stage). Additional invasive investigations and surgical diagnostic procedures or both had to be scheduled.

Table 3
Characteristics of patients with a Clinical or Pathological Diagnosis of Malignancy

Age (y) Mean (Range)	67.9 (41-88)	
	N(%)	
Sex		
Male	145 (67)	
Female	70 (33)	
		Percentage of subtotal
NSCLC Stage		
Stage I	26	16
Stage II	23	14
Stage III	47	30
Stage IV	54	34
Multiple synchronous[§]	9	6
Subtotal	159 (74)	
		Percentage of subtotal
SCLC		
Limited Disease	10	34
Extensive Disease	19	66
Subtotal	29 (13)	
Mixed Tumour (SCLC/NSCLC)	0	
Metastatic Disease	13[‡] (6)	
Other Primary Malignancies	14[†] (7)	
Total	215	

§ : 8 patients with synchronous lung cancers and 1 patient both a malignant lymphoma and a non-small cell lung carcinoma were diagnosed
‡ : 4 colorectal, 2 urothelial, 2 melanoma, 2 renal cell, 1 breast and 1 prostate metastasis
† : 4 malignant lymphoma, 2 thymoma, 2 mesothelioma, 5 large cell neuroendocrine carcinoma and 1 esophagus carcinoma

NSCLC, non small cell lung cancer; SCLC, small cell lung cancer

Table 4
Subtypes of NSCLC (Pathological and Clinical)

Subtype	Number(%)
Squamous cell carcinoma	58 (36)
Adenocarcinoma	73 (46)
Large cell carcinoma	4 (3)
Undifferentiated NSCLC	1 (1)
BAC and BAC with adenocarcinoma	3 (2)
Basaloid carcinoma	1 (1)
Multiple synchronous	8 (5)
Pleomorphic carcinoma	0 (0)
Clinical diagnosis of NSCLC*	11 (7)
total	159

NSCLC,Non-small cell lung cancer; BAC, bronchioalveolar carcinoma

* :diagnosis based on imaging

Timelines For the patient with a diagnosis of malignancy conduction times of the diagnostic process until start of treatment were assessed. The median number of days between detection of the suspicious abnormalities and first consultation was 9.0 days in the study period (figure 3).
The median number of days between the first and final consultation visit (where patients were informed about their diagnosis including tumour stage and treatment strategy) was 7.0 days. Treatment started after a median of 7,5 days.

Numbers of investigations
In figure 4 the rates of invasive diagnostic tests and surgical diagnostic procedures applied in patients with malignancy are presented. In 2%(4) of patients analysed in the study program, no invasive tests were performed at all and the diagnosis was based on imaging (3 patients with metastatic disease from a non-pulmonary origin and 1 patient with extensively metastasized disease who refused further analysis). During the one-day diagnostic workup, in 53%(114/215) of the patients with malignancy, the final diagnosis and disease stage were obtained with just one invasive investigation. These investigations were bronchoscopies in 26%(56/215), EUS in 12%(26/215), EBUS in 2%(4/215) or percutaneous US-guided biopsies in13%(28/215) of patients. In 41%(88/215) of the patients a bronchoscopy was not

performed at all during the analysis. For patients who underwent a bronchoscopy, the tumour was centrally located in 52% and was peripherally located in 48% of patients. Two or more invasive non-surgical tests were applied in 33%(71/215) of patients in the study group.
A stage and diagnosis was obtained in 92%(198/215) of patients with a malignancy in the study group with non-surgical investigations but 8%(17/215) of patients required surgery to finalize the diagnosis (staging in 14 and subtyping of malignant lymphomas in 3 patients).

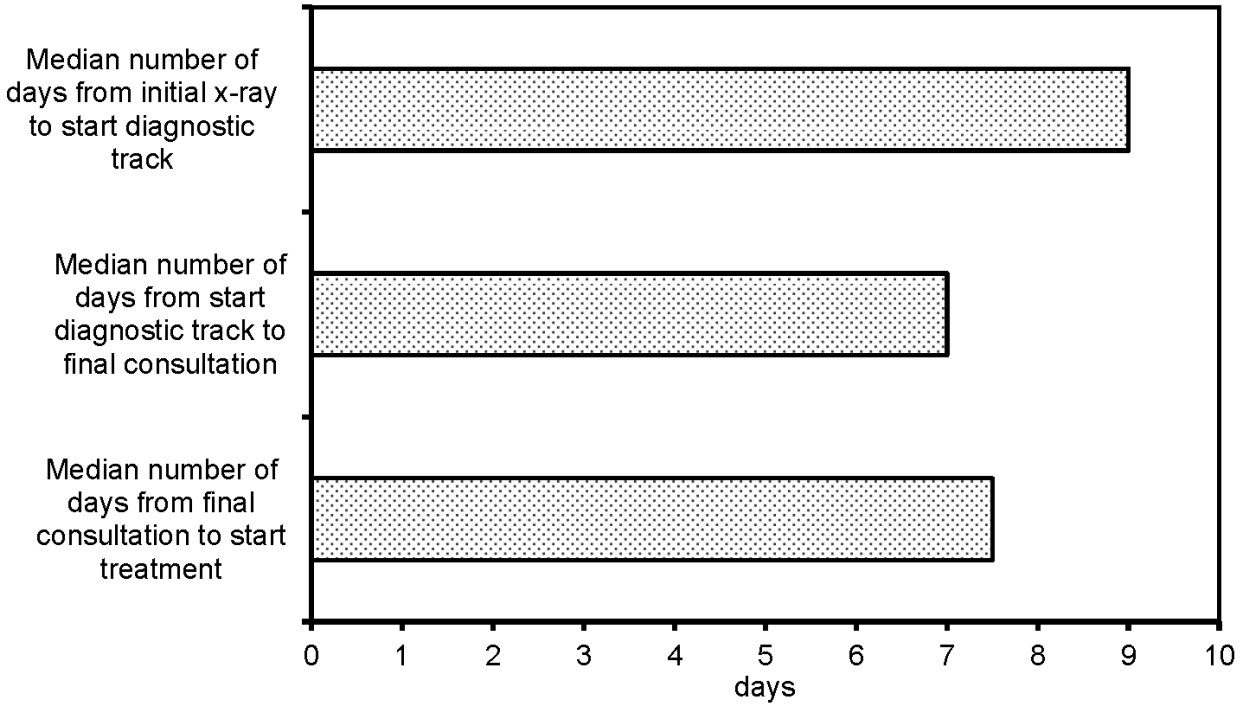

Figure 3. Patient waiting times during the diagnostic program for patients with malignancy.

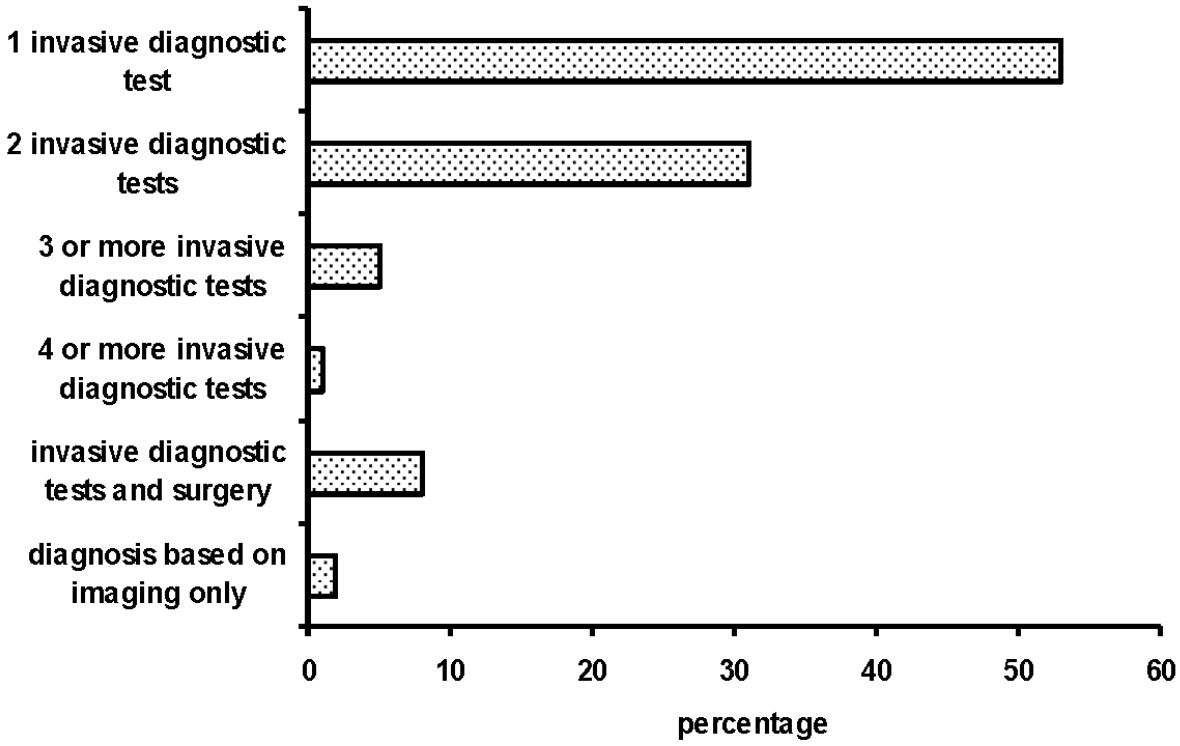

Figure 4
Rates of invasive diagnostic tests* and surgical diagnostic procedures§ applied to obtain a diagnosis and disease stage for patients with malignancy in fast-track diagnostic program (study group) and reference group.

*: Bronchoscopy, Ultrasound-guided percutaneous biopsies, Endoscopic Ultrasound-guided fine needle-aspirations, Endobronchial Ultrasound-guided transbronchial needle aspirations.
§: Surgical procedures study group: 11 mediastinoscopies, 3 lymph node biopsies, 1 VATS, 1 laparoscopy, 1 bone biopsy.

DISCUSSION

This paper describes the performance of a diagnostic program designed to improve the logistics of patients and diagnostic tools and to achieve a more sensible choice of invasive diagnostic investigations. Integrated PET-CT is the starting point for subsequent investigations to be performed on the same day. The location of biopsies is preferentially selected to obtain a tissue diagnosis as well as a verification of tumour stage.

Diagnostic results

Benign disease was diagnosed in 26% of patients which seems fairly high compared to 16% reported in a recently published study also with PET-CT as initial imaging modality in a fast track program[8]. A better selection could lower this figure. The additional metabolic information facilitated nevertheless the diagnostic process for

many patients with benign disease. We abstained for instance from further invasive diagnostics when lesions showed no FDG uptake.
In the vast majority of patients with a malignancy a pathologic diagnosis, including confirmation of tumour stage, was acquired on the diagnostic day of the program. The routine pathologic elaboration precluded immediate informing of patients the same day. From this perspective, our program differs from a fast track diagnostic program that was described recently providing a cytologic diagnosis at the end of the diagnostic day[8]. However, modern therapy is often customized to histologic subtyping and molecular characteristics. It is impossible to perform these refined techniques in a fast track (one-day) program although it is currently feasible to perform these advanced analyses on cytologic samples.

Diagnosing times
A short duration of diagnostic time, as one of the main goals of the study, was achieved. The time from first visit to consulting the patient about diagnosis, disease stage and treatment was just 7 days (including weekend days). This resembles the results of a Canadian study that demonstrated a reduction in diagnosing time compared to a retrospective preimplementation group. However state of the art diagnostics such as FDG-PET, endoscopic ultrasound and surgical staging procedures were not included in the workup of that study[9]. Moreover, it is not clear if determination of a final disease stage was part of the diagnosis.
Recommendations, stated in guidelines on waiting times, are met largely in our study group[14, 15].

Invasive diagnostic techniques
Another aim of this study was was to compress the diagnostic tests in one day, customized to the findings on the PET-CT. It was expected that a more adequate choice of diagnostic tools could lead to a low rate of invasive diagnostic tests. In this program there was a variety of immediately available US guided biopsy techniques all performed by pulmonologists. Primary tumours adjacent to the thoracic cage, supraclavicular or axillar lymph nodes and distant metastases were localized with percutaneous US and biopsied under direct vision. EUS-FNA and in a later phase also EBUS-TBNA was performed for mediastinal staging. Furthermore was EUS-FNA applied for upper abdominal staging. When imaging showed obviously extensive metastatic disease, the easiest accessible lesion was selected for biopsies according to the recommendations in a recent guideline of the American College of Chest Physicians (ACCP)[16].
In one third of patients, percutaneous or endoscopic US-guided needle aspirations in a metastasis or mediastinal lymph node, provided the final diagnosis and tumour stage at the same time. This observation supports our proposition that invasive non-surgical tests should be guided by the results of radiological and nuclear imaging. Corresponding to the Dutch Guideline on NSCLC, pulmonologists perform bronchoscopy as a standard procedure when lung cancer is suspected unless the tumour is smaller than 2 cm and has a peripheral location[15]. In other diagnostic programs bronchoscopy was the fixed invasive diagnostic modality after imaging with

CT and/or PET[8, 9]. This study demonstrates that bronchoscopy can be omitted in a substantial number of lung cancer patients when an appropriate choice from alternative techniques is made, providing not only a pathologic diagnosis but also a stage verification.

Limitations

Selection of patients was based on chest radiography in combination with clinical information in the referral letter. There were no predefined selection criteria. Unnecessary PET-CT's were made as a consequence of this diagnostic program and a cost analysis could elucidate the financial impact of this. To examine this properly a randomised controlled trial of initial PET-CT versus initial CT followed by the most appropriate diagnostic tests is required. It is possible however that patients with a benign outcome in the period before the program would have undergone relatively more unnecessary invasive diagnostic tests due to the absence of metabolic information provided by PET-CT. In patients in the study group with lesions showing no or very low FDG uptake, a wait-and-see strategy was followed and so invasive diagnostics could be spared. A clinical index of suspicion deduced from this study population or an extended population could help in a better selection of patients and consequently reduce the amount of redundant PET scans.

CONCLUSION

In almost all patients, a diagnosis (including tumour stage in patients with malignancy) could be made with a PET-CT and subsequent invasive diagnostic tests performed the same day. Integrated PET-CT as initial imaging modality is a guide to the lesions that provide a diagnosis and tumour stage at one time. These lesions can often be approached with percutaneous or endoscopic US-guided biopsy techniques. Therefore bronchoscopy is not per definition an obligatory part of the analysis of a suspicious abnormality.

CLINICAL PRACTICE POINTS

In reports on fast-track diagnostic programs for lung cancer subjects, CT and bronchoscopy are used as standard investigations. To provide a histologic diagnosis and a verified disease stage in that context is almost impossible but essential for treatment decisions. This study demonstrates that a proper choice of biopsy instruments, guided by PET-CT findings, results in a finalized analysis in almost all patients in one day. A diagnosis can be obtained by sampling lesions that provide a diagnosis and disease stage at once in many patients. Consequently time-consuming, unpleasant and unnecessary investigations can be prevented. Furthermore from this study it is obvious that bronchoscopy is not an obligatory part of the diagnostic strategy. After pathologic processing the patients can be informed about diagnosis and treatment options in a very short time.

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Chapter 9

Core biopsies versus fine-needle aspirations guided by endoscopic ultrasound procedures in enlarged mediastinal lymph nodes.

Submitted

Anthonie J van der Wekken, Jos A Stigt, Steven M Uil, James E Boers, Harry J Groen

ABSTRACT

Introduction

Endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) is an established way to examine mediastinal and upper abdominal abnormalities with cytologic specimen. EUS guided tissue core biopsies (EUS-TCB) provide samples for histologic analysis. In this prospective study we compared the diagnostic performance of both sampling techniques for pathologic and molecular analysis.

Methods

One hundred patients with pulmonary, mediastinal or left adrenal gland masses larger than 20mm in the sampling direction were analyzed with 4 passes of EUS-FNA followed by 4 times EUS-TCB. When biopsies were adequate, mutation analysis was performed.

Results

Sensitivities of EUS-FNA, EUS-TCB and both combined are 90.7%, 68.6%, and 96.5%, respectively. The difference between EUS-FNA and EUS-TCB is significant (P=0.00055). In the subgroup with malignancy, EUS-FNA is also significantly superior over EUS-TCB (P=0.00028).

Non-squamous cell carcinoma was diagnosed in 29 patients. In 3/29 patients no molecular analysis was performed. Mutation analysis was successful in 12/26 (46%) of biopsies and 14/26 (54%) of biopsies were inadequate for molecular analysis.

Conclusions

EUS-TCB has no added value over EUS-FNA for pathologic analysis of pulmonary, mediastinal or left adrenal masses. Tissue appeared inadequate for molecular analysis in more than half the patients.

INTRODUCTION

Endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) is widely used to diagnose pathologic mediastinal masses (upper left paraoesophageal, subcarinal and lower paraoesophageal sites) as well as the upper abdominal and left adrenal masses. High diagnostic performances of EUS-FNA were described for mediastinal lesions with reported sensitivities and specificities ranging from 78-85% and 92-100%, respectively, and diagnostic accuracies up to 95%[1-9].
With EUS-FNA, cytological samples are obtained that provide a diagnosis and stage of disease in most patients based on morphology and additional immunocytochemic staining of embedded cells. For optimal pathologic analysis however, assessment of the tissue structure might help in refining the diagnosis. Furthermore do histologic samples usually contain more tumor cells, providing more material for a molecular evaluation. Tissue is often obligatory for subtyping of some malignancies, like lymphoma and breast cancer[10, 11]
Endoscopic ultrasound guided tissue core biopsy (EUS-TCB) needles were developed to provide small tissue samples enabling evaluation of tumor cells in their tissue context. In gastroenterology EUS-TCB was shown to be useful in the evaluation of benign and malignant disease in the upper abdomen[7, 12-23] In 2 retrospective studies the diagnostic performance of EUS-TCB was described for thoracic lesions[24, 25]. For EUS-TCB in mediastinal lesions a diagnostic accuracy of 90% was reported[24]. In combination with EUS-FNA the accuracy increased to 98%[25].
Complication rates for EUS-TCB were not higher than for EUS-FNA (2% for both in transgastric use[19]).
Studies on the appropriateness of EUS-TCB for molecular analysis are not available. Therefore we performed a prospective study comparing the results of EUS-TCB with EUS-FNA. Our primary objective was to investigate whether EUS-TCB has added value over EUS-FNA alone for the diagnosis of mediastinal or left adrenal masses. A secondary objective was to study whether EUS-TCB would provide tissue enabling molecular analysis adequately in patients with a diagnosis of non-squamous cell carcinoma of the lung.

MATERIALS AND METHODS

Patient selection

Patients with mediastinal masses or enlarged left adrenals of at least 20 mm (as measured on CT) entered the study after informed consent was obtained. For primary tumor and adrenal gland the largest diameter was used and for lymph nodes the short axis diameter was used**.**
The study was performed in the Isala Klinieken, Zwolle, the Netherlands and approved by the local medical ethical committee.

Sampling procedures

Four fine-needle aspirations followed immediately by intentionally four tissue core biopsies were performed in masses measuring at least 20 mm in the biopsy direction on ultrasonography(the needle extends 20 mm when biopsies are taken). When the TCB needle failed before 4 biopsies were taken, the number of performed biopsies was noted.

EUS was performed by using a Pentax Hitachi linear endoscope (FG-36UX, diameter 2,4mm and EG-3830UT, diameter 3,8mm). Fine-needle aspiration was performed with an Echotip Endoscopic ultrasound needle of 22 G (Cook Ireland Ltd).

Cytologic material was deposited on two air-dried slides and in Saccomanno's fluid. Biopsies were performed using the 19G Quick core endoscopic ultrasound biopsy needle (Cook Ireland Ltd) and collected in formalin 4% (Figure 1). EUS-TCB and EUS-FNA was performed by two experienced endoscopists.

Pathologic assessment

Of each of the EUS-FNA samples, 2 air-dried slides were Giemsa stained and assessed by an experienced pathologist. The rest of the sampled material was collected in Saccomanno's fluid (50% v/v ethanol & polyethylene glycol 1500) for the preparation of cell paraffin blocks. Four 10-micron slides were cut from the blocks on 3 levels (approx. 250 microns apart) and stained with hematoxylin and eosin (H&E). If >50 tumor cells were found in the H&E slide, additional immunohistochemical staining was performed using thyroid transcription factor-1 (TTF1), cytokeratin 7, p63 and chromogranin A in addition to alcian blue stain.

Core tissue biopsy material was collected in 4% formalin, and fixed overnight. Paraffin blocks were prepared; slides were cut and stained in the same manner as the cell paraffin block material. If a metastasis from other primary sites than lung was considered, appropriate additional immunohistochemical stains were ordered.

Molecular analysis

When non-squamous cell carcinoma of the lung was diagnosed, the TCB samples were assessed for molecular analysis. Adequate samples were cut into five 10-micron slides, DNA was extracted[26], and high resolution melting analysis (HRM) was performed for KRAS exon 2 and Epidermal Growth Factor Receptor (EGFR) exons 18, 19, 20 and 21. An abnormal HRM curve was followed by Qiagen pyrosequencing for confirmation and detection of the exact nature of KRAS or EGFR mutation.

Figure 1

The upper panel shows the ultrasound image of a tissue core needle biopsy (19G) in a left adrenal metastasis.

The middle panel shows the corresponding CT image showing enlarged adrenals.

The lower panel shows the Quick core endoscopic ultrasound biopsy needle (19 G Cook Ireland Ltd) closed (above) and opened (below).

Statistical Analysis

Given an alpha of 0.05 and a power of 80% the minimum required total sample size is 98 patients to detect an increased sensitivity from 74% for FNA to 94% for TCB. Differences in sensitivity, specificity and accuracy between the diagnostic modalities have been tested with the McNemar's test for correlated proportions[27]. P-values <0.05 were considered significant. Analyses were performed using SPSS-Statistics version 20.0 (IBM corporation, Armonk, NY, USA).

RESULTS

Between August 2009 and February 2012, 100 patients entered the study. Patient characteristics are described in table 1. In 15 patients the EUS-TCB needle failed before 4 biopsies were taken (Table 1).

Table 1

Patient characteristics of 100 patients with enlarged mediastinal lymph nodes or enlarged adrenal glands.

Age (mean)	22-84 (60)
Male/ Female	70/30
Site of intervention	**N**
N2	1
N4L	5
N7	81
N8	3
Left adrenal gland	4
Primary tumor	6
Diameter on CT	
Tumor/ Left adrenal gland (largest axis)	25-83 mm
Lymph node (short axis)	11-38 mm
Needle passes	**N**
FNA	100 x 4*
TCB	1 x 1**
	5 x 2
	9 x 3
	85 x 4

* 100 patients had 4 needle passes

** 1 patient had one biopsy, 5 patients had 2 biopsies etc. due to needle

There were no complications observed after performing EUS-FNA and EUS-TCB sequentially.

Diagnostic performance

All diagnoses are summarized in table 2. Half of the patients had a pulmonary malignancy; about one third had benign disease.

Table 2

Diagnosis established by EUS-FNA, EUS-TCB or both.

Final diagnosis	No. patients	EUS-FNA	EUS-TCB	EUS-FNA + TCB
Correct diagnosis	100	88	66	95
Lung malignancy	51	49 (96%)	34 (67%)	50 (98%)
NSCLC	38	36 (95%)	24 (63%)	37 (97%)
Non-squamous	29	24 (83%)	17 (59%)	28 (97%)
Mutation analysis	22	19 (86%)	12 (55%)	22 (100%)
SCLC	13	13 (100%)	10 (77%)	13 (100%)
Lymphoma	3	3 (100%)	3 (100%)	3 (100%)
Other malignancy*	7	7 (100%)	7 (100%)	7 (100%)
Granulomatous Disease	25	19 (76%)	15 (60%)	23 (92%)
Benign**	14	11 (79%)	7 (50%)	12 (86%)
Incorrect diagnosis		12	34	5
False diagnosis***		6	2	1
Inadequate******		6	32	4

*: Renal cell carcinoma, rectal carcinoma and melanoma, **: Reactive and normal lymph node, ***: including sample errors, ****: including dry tap and uncertain diagnosis.

A definitive diagnosis was obtained by EUS (n=84), mediastinoscopy (n=2) or broncheoalveolar lavage (n=1). In 14 patients mediastinal lymphadenopathy was considered reactive, based on test results in combination with clinical follow up. One patient with lymphoid tissue in EUS-FNA was diagnosed as sarcoidosis based on bronchoalveolar lavage results and radiologic appearance. In one patient, EUS-FNA and EUS-TCB were inadequate and uncertain respectively. The patient died of disseminated melanoma without radiologic suspicion of mediastinal involvement.
Table 3 shows the diagnostic performances of EUS-TCB and EUS-FNA. The sensitivities of EUS-FNA and EUS-TCB are 90.7% (95%CI: 82.7;95.2), respectively 68.6% (95%CI: 58.2;77.4). The difference is significant (P = 0.00055; 95%CI: 10.7;33.4). The sensitivity of the combined procedure is 96.5% (95%CI: 90.2;98.8). The diagnostic accuracy for EUS-FNA, EUS-TCB and both combined is 91.0%, 73.0% and 96.0% respectively. The difference in diagnostic accuracy is significant (P<0.01; 95%CI: 5.6;30.4).
In malignant disease (N=61) sensitivity of EUS-FNA, EUS-TCB and both combined is 96.7% (95%CI: 88.8;99.1), 72.1% (95%CI: 59.8;81.8), and 98.4% (95%CI: 91.3;99.7) respectively (table 3). Difference between both FNA and TCB for determining malignant disease is significant (P = 0.00028; 95%CI: 12.9;36.3).
In the subgroup of patients with granulomatous disease (N=25) sensitivity of EUS-FNA, EUS-TCB and both combined is 76.0% (95%CI: 56.6;88.5), 60.0% (95%CI: 40.7;76.6) and 92.0% (95%CI: 75.0;97.8) respectively. Difference between EUS-FNA and EUS-TCB for determining granulomatous disease is not significant (P = 0.39; 95%CI: -10.4;42.4).

Table 3
Operating characteristics of EUS-FNA, EUS-TCB and both.

	sensitivity			**specificity**	**accuracy**
	Overall	**Malignant disease**	**Granulomatous disease**	**Overall**	**Overall**
	N=100	N=61	N=25	N=100	N=100
EUS-FNA	90.7%	96.7%	76.0%	92.9%	91.0%
EUS-TCB	68.6%*	72.1%**	60.0%	100.0%	73.0%
EUS-FNA + EUS-TCB	96.5%	98.4%	92.0%	92.9%	96.0%

* p=0,00055 EUS-FNA vs. EUS-TCB, **p=0,00028 EUS-FNA vs. EUS-TCB.

Molecular analysis

In three out of 29 patients with non-squamous carcinoma, biopsies were not assessed for molecular analysis for unknown reasons. In 12 biopsies,of 26 patients with non-squamous cell carcinoma, tumor cellularity was above 10% with good DNA quality and so molecular tests followed. Inadequate material was found in 14/26 (54%). The diagnostic success rate was 46%. In 8/14 patients with inadequate biopsies, mutation analysis was performed on FNA successfully. In 5/14 patients with inadequate biopsies, no mutation analysis was performed on FNA and in 1/14 patients with inadeqate biopsies, also the FNA was inadequate for molecular diagnostics.

In 19/29 patients mutation analysis was performed on aspirated samples. In 1/29 patients the material was inadequate. The diagnostic success rate was 19/20(95%). In 8/29 patients mutation analysis was not performed (in 3/8 patients mutation analysis was already performed on TCB and in 5/8 there was no clinical indication).

Performance of biopsy device

In 15 patients the EUS-TCB needle failed before 4 biopsies were taken (Table 1). Failure of needles means that the needle could not be tightened again for subsequent biopsies. In 6/15 patients with 1 or more needle failures a diagnosis could not be made on the available samples. In 33/85 patients with all 4 biopsies a diagnosis could not be made.

DISCUSSION

Performance characteristics

This prospective study did not demonstrate additional value of EUS-TCB (expressed as a higher sensitivity) over EUS-FNA alone in the analysis of mediastinal and adrenal masses, unlike previous retrospective studies[24, 25].

The diagnostic accuracy of 73.0% in our study for EUS-TCB contrasts to the accuracy of 90% reported before[24]. The diagnostic accuracy of 96.0% of the combination of EUS-TCB and EUS-FNA in our study matches the diagnostic performance of 98% reported in a previous study[25].

A procedural cause for this inferior accuracy cannot be found. FNA and TCB was performed in the same order although up to 6 biopsies were obtained in both previous studies[24, 25]. In one study the diversity of diagnoses was comparable to this study[25]. In the other study, performed in a referral centre for granulomatous disease, there was a preponderance of granulomatous disease[24].

In this study, the pathologist was unable to diagnose biopsies adequately in a high proportion of patients compared to cytologic samples. This could be explained by the characteristics of samples obtained with both procedures. In TCB, the maximum biopsy size is theoretically 20 by 0.9 mm but in practice smaller. Moreover, TCB contains often fibrous and/or necrotic tissue precluding a confident diagnosis. The FNA samples often contain a mixture of 'micro-biopsies' of fibrotic tissue and aspirated separate cells as can be clearly seen in cell block slides.

Variation in the direction of sampling is smaller with the more rigid TCB needle compared to the FNA needle that enables sampling of a greater proportion of the lymph node by multiple passes in different directions.
Another reason for inferior performance of TCB is failure of needles. We observed needle failure in a substantial number of patients before the preplanned number of 4 biopsies was reached. This contrasts to the FNA needle that allowed always 4 passes in every patient. So, overall, less TCB than FNA was performed in the total group. In 6/15 failures it contributed to a worse clear diagnosis.
As in previous studies no complications were seen in performing EUS-FNA and EUS-TCB sequentially in one session[19, 24].

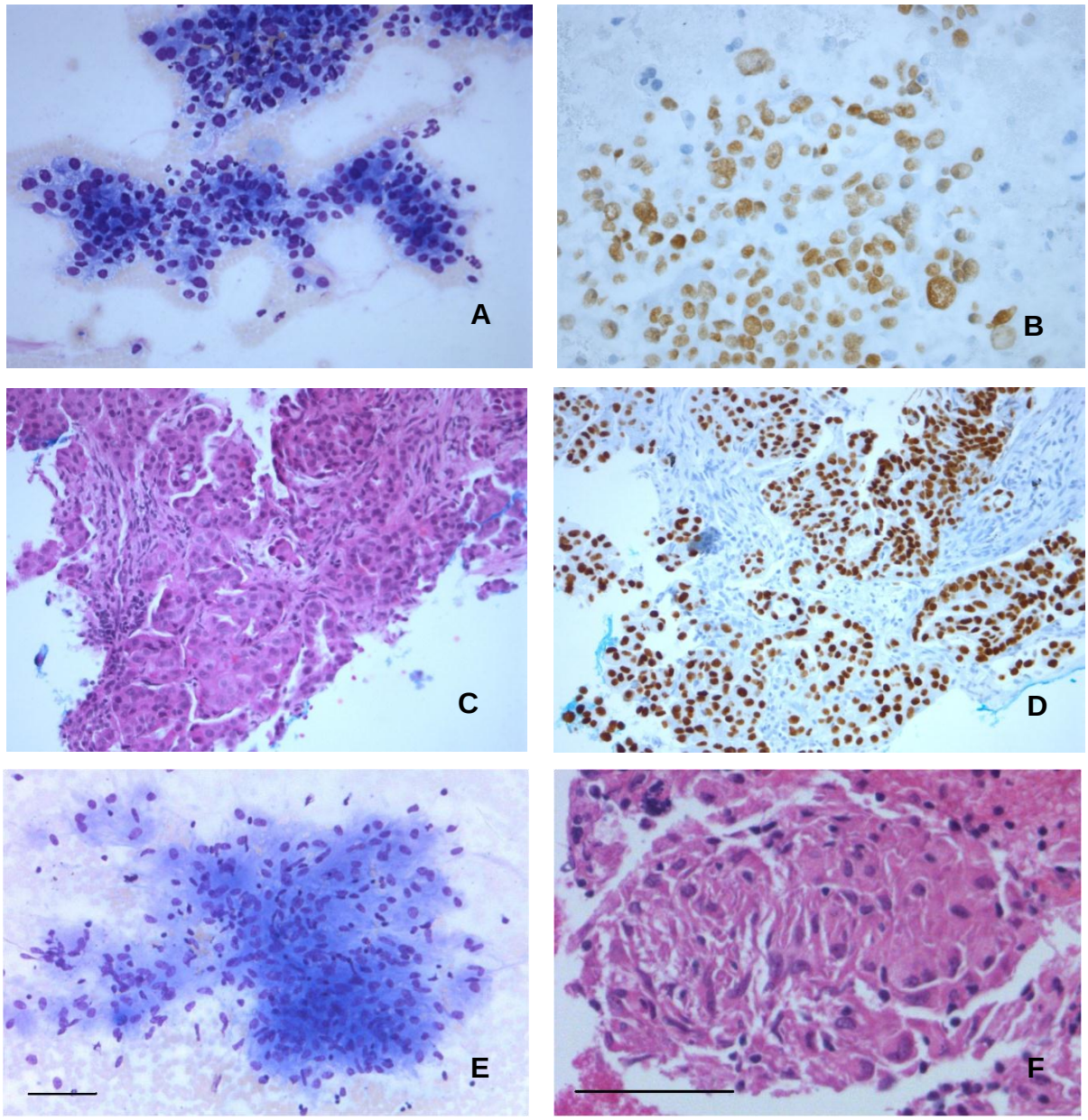

Figure 2. EUS-FNA with (A) Giemsa stained smear and (B) cell block with TTF1-immunohistochemistry showing positive nuclei. Corresponding quick core biopsy with (C) H&E and (D) TTF1 stains. All show adenocarcinoma. Granuloma in smear (E) as well as in Agar block (F). Original magnification: 40x. Bar length 100 micron.

Performance characteristics in subgroups

EUS-FNA was significantly more sensitive than EUS-TCB for the subgroup of malignant disease. Although in tumor subtyping histology is still the golden standard, an adequate diagnosis is also possible with immunohistochemical staining of cytologic samples (figure 2). So, not only the interconnective structures but also the volume and quality of samples is of importanceIn this study we observed that in experienced hands EUS-FNA and EUS-TCB do not differ with respect to amount of tumor material obtained. In patients with granulomatous disease a difference between EUS-FNA and EUS-TCB was not obvious but the group was small. In this subgroup we often experienced a low to even absent yield in TCB samples. The solid rubbery texture in many granulomatous nodes is a possible explanation for the often very small or empty biopsies.

It was disappointing to observe that EUS-TCB had no additional value in 6 patients with malignant lymphoma as for this diagnosis histologic samples are almost invariably necessary for further subtyping.

Molecular analysis

TCB often contained insufficient material (or no material at all) for molecular analysis in more than half of patients. This contrasted with the high diagnostic success rate of mutation analysis in FNA. There was only one patient with a failing molecular analysis on FNA but it failed on biopsies as well. The high diagnostic success of mutation analysis in EUS-FNA is supported by several recent studies[28-30]. Consequently, there is no need to obtain EUS-TCB for mutation analysis.

Limitations

Our study on the diagnostic performance of EUS-TCB is probably influenced by needle failures and the fact that core biopsies of 19 Gauge are still small. Refining the needles in order to improve repeated biopsies, enlarging the needles to produce larger samples or developing other techniques like ProCore[31], where larger structures of histologic material can be obtained, may result in better outcomes.The subgroups of granulomatous disease and malignant lymphoma were too small for an adequate analysis of the performance of EUS-TCB versus EUS-FNA.

Also for the comparison of the appropriateness of both sampling techniques for molecular analysis, larger groups should be analyzed and biopsies as well as FNA should be compared in all patients.

CONCLUSION

In conclusion, although the technique of the EUS-TCB by Quick Core is safe, it has no added value over EUS-FNA in diagnosing mediastinal or left adrenal masses, particularly in malignant disease in experienced centers.

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Chapter 10

Pyrosequencing analysis of *EGFR* and *KRAS* mutations in EUS and EBUS derived cytologic samples of adenocarcinomas of the lung.

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Jos A. Stigt, Nils A. ‘t Hart, Ageeth J. Knol, Steven M. Uil, Harry J.M. Groen

ABSTRACT

Introduction: Patients with stage IV non-small cell lung cancer (NSCLC) harbouring an activating epidermal growth factor receptor (*EGFR*) mutation are eligible for treatment with *EGFR* tyrosine kinase inhibitors. With pyrosequencing, low-frequency mutations may be detected more easily even in small diagnostic samples like endoscopic or endobronchial ultrasound guided needle aspirations (EUS-FNA and EBUS-TBNA). The diagnostic performance of pyrosequencing in analysing cytological specimens is compared with the routinely used high resolution melting (HRM) and Sanger sequencing.
Methods: Patients diagnosed with adenocarcinoma of the lung were selected from a FNA and TBNA specimen database. If formalin fixed paraffin-embedded tumor blocks were available, mutation analysis was performed for *EGFR* and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) genes using both pyrosequencing and HRM. When HRM showed abnormalities, Sanger sequencing was used.
Results: A total of 126 samples were available for mutation analysis. The analysis success rate for pyrosequencing and HRM were 97% and 93%, respectively. HRM failures were observed in fragmented DNA showing chains of 100-200 base pairs (bp). A significant correlation between length of DNA fragments (100-300 versus 300-400 bp) and mean sample age (797 days versus 317 days) was found ($P<0.0001$) suggesting an influence of sample age on DNA quality.
Conclusion: Pyrosequencing on cytological blocks, especially older tumor blocks, is feasible with a high diagnostic success rate. Failures in HRM were observed in DNA samples with short fragments related to longer storage times.

INTRODUCTION

A subset of the pulmonary adenocarcinomas harbouring mutated epidermal growth factor receptor (*EGFR*) genes have a prolonged survival irrespective of treatment[1]. Mutations cause alterations in the intracellular part of the transmembrane *EGFR*. This results in a stronger binding of tyrosine kinase inhibitors (TKIs) than ATP with subsequent inhibition of *EGFR*[2]. When compared to chemotherapy, treatment with *EGFR*-TKIs showed higher response rates and significant better progression-free survival with mild toxicity[1, 3-6].
This benefit in patients with activating *EGFR* mutations requires mutation analysis as a standard diagnostic procedure in patients with stage IV adenocarcinoma of the lung.
Another even more frequently encountered mutation in adenocarcinomas of the lung occurs in the V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene.
Recommendations on tissue and test characteristics for *EGFR* mutation analysis were recently published in an European consensus report[7]. The authors prefer the use of histology and mention the possible mutation analysis in cytological specimens. In reported large clinical studies, mutation analysis is almost invariably performed on tissue samples.
In routine clinical practice tissue samples are required, however, a diagnosis is often made on cytological specimens. Tissue biopsies are not always available. Therefore, mutation analysis of small tumor samples is necessary. Mutation analysis on cytological specimen, including transbronchial and transesophageal aspirates, is reported previously in several studies[8-20]. This is particularly relevant for the majority of stage III patients progressing to stage IV, enabling archived mediastinal aspiration cell blocks to be used for molecular processing. Those aspirates can be performed in the same lymph node in different directions gathering multiple aspirates from different parts of the same node metastasis thereby increasing the yield of tumor cells.
Although it is not clear which method is the best for mutation analysis in NSCLC, a combination of DNA amplification and direct sequencing is the most practiced.
After DNA extraction, amplification of target sequences with the polymerase chain reaction (PCR) is the next step. Prescreening for abnormal target alleles is possible with high resolution melting (HRM)[21]. The sensitivity of detecting mutations can be increased by amplification refractory mutation system (ARMS-PC)[22], allele specific PCR[23, 24], peptide nucleic acid (PNA) clamping methods[25, 26] or preferential amplification of mutant alleles with co-amplification at lower denaturation temperature-PCR technique (COLD-PCR)[20].
When genomic abnormalities are detected, further characterization with DNA sequencing follows. Various techniques for sequencing have been developed. Test characteristics improved over time and more recently developed tests require less tumor cells.
The question remains whether screening should precede sequencing or whether sequencing should be performed upfront. The diagnostic accuracy of tests in relation

to the quality of samples as well as the costs and diagnostic speed are important factors in designing the optimal testing strategy.
Pyrosequencing is one of the latest assays using luminometric instead of electrophoretic detection[27]. This technique enables characterization of mutations and quantification of mutated alleles in samples with low tumor cell density and detection with high accuracy rates[28].
The objective of this study is to compare the diagnostic performance of pyrosequencing with the comprehensive strategy of HRM (followed by Sanger sequencing[29] in case of abnormalities) for *EGFR* and *KRAS* mutation analysis in paraffin-embedded cytological specimens of adenocarcinoma patients obtained with EUS and EBUS. The analysis success rates and concordance rates for both techniques are determined.

PATIENTS AND METHODS

Patients

Patients with a cytological diagnosis of adenocarcinoma established on EUS or EBUS derived samples were selected from our local patient database. The diagnosis was based on morphologic and immunohistochemical characteristics for all tumors. All samples were coded and managed independently. The study was approved by the Medical Ethical Committee of the Isala Clinics in Zwolle, the Netherlands.

EUS and EBUS

EUS and EBUS was performed with Pentax ultrasound endoscopes (EUS FG-36UX respectively EBUS EB-1970UK; Pentax, Tokyo, Japan) with a Hitachi EUB-5500 processor (Hitachi, Tokyo, Japan). The fine needle aspirations (FNA) were performed under conscious sedation with midazolam and with local anaesthesia sprayed in the oropharynx (lidocain 1%) and lidocain gel 20 mg/ml. Needles of 22-Gauge were used for sampling and at least two aspirates were smeared on slides initially. Aspirations per site (3-4 passes in different directions of the tumor or enlarged mediastinal lymph node) were performed and deposited in carbowax 2% fixative. Cell blocks were made using cell pellets embedded in AGAR 10%, followed by formalin fixation, dehydration and paraffinization.

Tumor cell density estimation

Sections were cut from formalin fixed paraffin-embedded (FFPE) tissue blocks. The first and last sections were stained with haematoxylin & eosin (H&E) for histopathological examination. The percentage of tumor cells is estimated using the first and last HE section. The estimated ratio is based upon the tumor cell amount compared to stromal cells and lymphocytic background.

DNA extraction

Genomic DNA was extracted from the remaining sections. The sections used for DNA extraction were deparaffinated and genomic DNA was extracted using the

QIAamp DNA FFPE Tissue Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's instruction. The extracted DNA was eluted in 100 µl ATE buffer. DNA quality was checked by multiplex ladder PCR[30]. 3µl Mastermix (500 µl 10x Gold buffer, 40 µl 100 mM Gene Amp dNTP Blend, 250 µl Glycerol 87%, 50 µl Cresol Red, 300 µl 25 mM $MgCl_2$, 360 µl double-distilled H2O (ddH_2O)); 5 µl primermix; 0,08 µl Amplitaq Gold (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands); 200 nM of each primer; 1,0 µl ddH_2O and 1 µl sample per test were used. Primers used are listed in e-Table 1. PCR cycling was performed on a Veriti 96-well Thermal Cycler (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands) using the following conditions, one 12-minute cycle at 94,4°C was followed by 30 cycles of respectively 30 sec at 95°C, 30 sec at 59°C, 45 sec at 72°C and finally one cycle of 5 min at 72°C. The produced DNA amplicons were separated using agarose gel electrophoresis (3% agarose gel, 200 volt for 1 hour) and assessed with UV light.

Mutation analysis

Exons 18, 19, 20 and 21 of the *EGFR* gene and exons 2 and 3 of the *KRAS* gene were examined with HRM as previously described[21, 31]. HRM was performed in a total volume of 10 µl, containing 4 µl LightScanner mastermix (Idaho Technology Inc. Salt Lake City, Utah, USA), 2 µl genomic DNA, 2 µl 100 nM of each primer and 2 µl ddH_2O. PCR cycling and HRM analysis were performed on a LightCycler 480II (Roche Diagnostics, Almere, the Netherlands) according to conditions previously described[32]. When the HRM plots were abnormal HRM amplicons were checked with Sanger sequencing. HRM products were purified using ExoSAP-IT (GE Healthcare, Hoevelaken, the Netherlands) according to the manufacturer's instruction. Primers used are listed in e-Table 2.

The purified HRM products were used for Sanger sequencing using the Big Dye Terminator v3.1 kit (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands). The reaction mix consisted of 2 µl Sequencing RR-100, 3 µl 5x Sequencing buffer, 4µl ddH_2O, 1 µl purified PCR product and 10 µl 2.5 µM M13 primer in a final volume of 20 µl.

The sequence reaction was run on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions, one cycle of 96°C for 1 minute followed by 25 cycles of 96°C for 10 sec, 60°C for 125 sec and at 4°C subsequently. The products were purified using the DyeEx 2.0 Spin Kit (Qiagen) according to the manufacturer's instructions and run on a 3130 Genetic Analyzer (Applied Biosystems). Afterwards the sequences were analyzed using the Sequencer 3.0 software (Applied Biosystems).

Pyrosequencing was performed using the PyroMark Q24 (Qiagen). The therascreen *EGFR* and *KRAS* Pyro Kits (Qiagen) were used according to the manufacturer's instruction. The targeted sequences of *EGFR* and *KRAS* were amplified using PCR (Veriti 96-well Thermal Cycler, Applied Biosystems) using the following conditions, one 15 minute cycle at 95°C followed by 42 cycles of respectively 20 sec at 95°C, 30 sec at 53°C, 20 sec at 72°C and finally one cycle of 5 min at 72°C. Each PCR product was used as a template. The sequencing primer hybridizes close to the sequence of interest. Pyrosequencing was performed using PyroMark Gold Q96

reagents (Qiagen) containing enzyme and substrate mixture, dATP-S, dCTP, dGTP and dTTP. Nucleotide incorporation is followed by release of ATP. Luciferin and ATP generates light emission following a reaction catalyzed by luciferase. The unique dispensing order described by the manufacturer is used to detect possible mutations in the targeted sequence. The pyrogram is analyzed using the pyrosequencing data analysis software (Qiagen).

Costs of materials and handling time

The costs of materials to run a single sample for HRM, Sanger sequencing and pyrosequencing are 100$, 355$ and 700$, respectively. If possible, costs can be reduced by analysing up to two patient samples per run for EGFR exon 18-21 and KRAS exon 2-3 (520$ per patient for two cases including blanc controls). The time required for a laboratory technician to perform a single HRM analysis is 50 minutes. When Sanger sequencing follows HRM the total handling time is 90 minutes. Pyrosequencing takes 100 minutes and the total time required for HRM followed by pyrosequencing is 150 minutes.

Statistical analysis

Patient characteristics were examined with descriptive statistics. A Pearson correlation coefficient was used to assess the association between estimated tumor density and allele frequency. The difference in mean sample age between base pairs (bp) categories 100-300 and 300-400 was analyzed using an unpaired Student's t-test.

RESULTS

Patient samples

Between June 2008 and September 2011, pulmonary adenocarcinoma was diagnosed in 169 patients by different pathologists using EUS-FNA (N=90) or EBUS-TBNA(N=79).
After reviewing the slides for study purposes the diagnoses changed to a different pathologic classification in five patients. For all other patients (N=164) there was an inquiry for adequate tumor material in deposit. From 24 patients no agar embedded material was stored and from 14 other patients no DNA was available (figure 1).
In six patients DNA had been isolated previously and was not available anymore. FFPE tissue blocks did not contain material to re do the DNA extraction after the first extraction and immunohistochemical analysis. HRM had been performed in the samples of all of these patients followed by Sanger sequencing in three cases. There were five EGFR and KRAS wild type and one EGFR exon 19 deletion.
Tumor samples of 126 patients were processed for DNA analysis. Patient characteristics, including disease stages, are described in table 1.

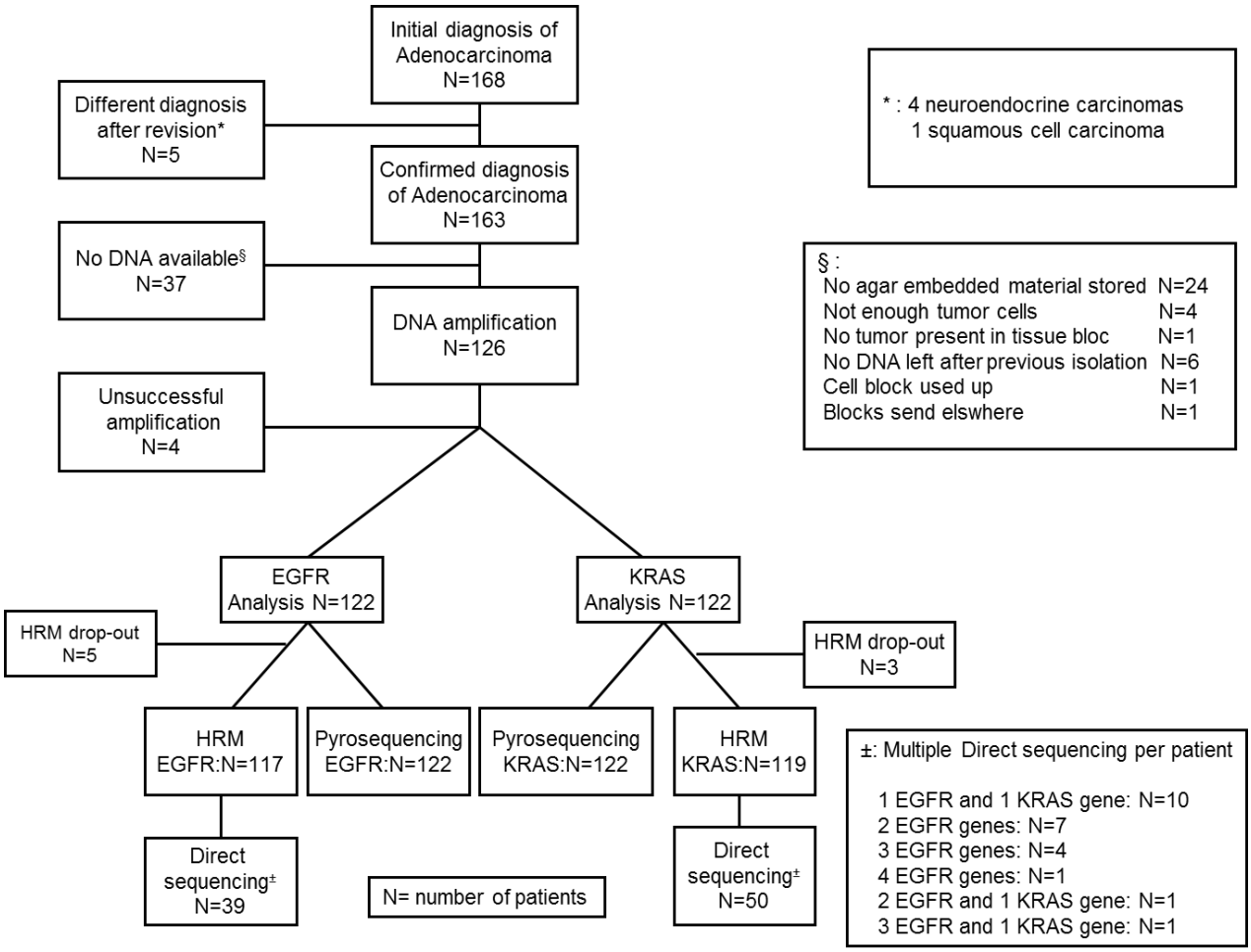

Figure 1 Flow chart showing the selection of patient samples and the successive stages of processing and analysis performed on them

Amplification was not successful in 4/126 patients resulting in 122 samples that were further analysed with both HRM and pyrosequencing.
Due to insufficient DNA quality in five samples, HRM was unsuccessful (HRM for both *EGFR* and *KRAS* failed in 3 patients and HRM for *EGFR* alone failed in 2 patients).
In 39/117 (33%) patients, HRM showed abnormalities in the *EGFR* exons and in 50/119 (42%) patients, HRM showed abnormalities in the *KRAS* exons. In these patients direct sequencing was performed to confirm or exclude mutations. In 24 patients *EGFR* as well as *KRAS* sequencing was performed due to abnormalities in both gene sets.

Mutations
Table 2 shows the results of pyrosequencing for *EGFR* and *KRAS* in EUS and EBUS samples. In 122 samples of adenocarcinoma, 15(12%) EGFR mutations (12 activating and 3 inhibiting mutations) and 51(42%) KRAS mutations were detected.

Table 1
Characteristics of 126 patients eligible for DNA processing

Age ,average (range), yr	66(26-85)
Gender	
Male	65
Female	61
EUS : EBUS	63:63
Tumor stage	
Stage 1b	2
Stage 2a	4
Stage 2b	2
Stage 3a	63
Stage 3b	12
Stage 4	43
Mean sample age in days (range)	585(2-1323)
Mean tumor cell content (range)	30% (5-90)

Detection rate and tumor analysis success rate
The detection rate of pyrosequencing for *EGFR* and *KRAS* mutations was 122/122(100%) and the tumor analysis success rate was 97%(122/126) for both genes.
The detection rate for *EGFR* mutations in all endosonography guided samples with HRM in combination with Sanger sequencing was 117/122 (96%) and the tumor analysis success rate 93% (117/126).
Sanger sequencing for *EGFR* and/or *KRAS* genes following HRM was performed in 76/119 samples showing single-nucleotide polymorphisms (SNP) in 19 samples when HRM was abnormal for exons 18(N=11) and 21(N=8). Detection of SNP's is relatively high due to the chosen primer sets which include a common SNP18 hotspot. The SNP rate for exon 18 could be reduced by using different primer sets.
For *KRAS* the analysis success rate was 94%(119/126). Abnormalities detected with HRM in codon 12, 13 and 61 were all based on mutations.

Concordance rates
For *EGFR* mutation analysis, HRM with Sanger sequencing in case of HRM abnormalities and pyrosequencing showed a concordance rate of 100%(117/117).
For *KRAS* mutation analysis the concordance rate was 98%(115/117). In two

patients pyrosequencing revealed a G12C point mutation that was not discovered with HRM.

Tumor cell density

The mean estimated tumor density in samples with mutations was 35.5% (range 5-90%). The mean frequency of the mutated alleles provided by the pyrosequencing software was 38.7% (range 5.7-89.3%). The estimated tumor density and the frequency of mutated alleles were well associated (Pearson correlation coefficient of 0.57 (P<0.001).

Factors influencing test results

The quality of DNA, expressed as the length of base pairs (bp) was studied with agarose gel electrophoresis of PCR products. Shorter DNA chains indicate more fragmentation. All patients were classified into two subgroups with DNA of 100-300 bp and 300-400 bp showing mean sample ages of 784 days and 354 days, respectively (P<0.0001). All five HRM failures showed short DNA fragments (100-200 bp).

The estimated tumor cell density in five samples, that proved to be inadequate for HRM analysis, was 5% (n=1), 10% (n=1), 30% (n=2) and 50% (n=1). In the four samples with an amplification failure, the estimated tumor cell densities were 5%(n=1), 10%(n=2) and 60%(n=1).

Table 2

Mutations found in EUS and EBUS guided fine-needle aspirations of 122 patients with pyrosequencing technique.

EGFR mutations			**KRAS mutations**		
		Number(perc)			**Number(perc)**
Exon 19	delE746-A750	4 (27%)	Exon 2	G12C	24 (47%)
Exon 19	delL747-P753insP	1 (7%)	Exon 2	G12A	8 (16%)
Exon 20	T790M	3 (20%)	Exon 2	G12V	7 (14%)
Exon 21	L858R	7 (47%)	Exon 2	G12D	6 (12%)
			Exon 2	G12R	1 (2%)
			Exon 3	G13C	1 (2%)
			Exon 3	G13D	3 (6%)
			Exon 3	Q61H	1 (2%)
Total		15			51

DISCUSSION

In this study, the diagnostic performances of two sequencing strategies were compared on cytological samples from mediastinal lymph node metastases in patients with adenocarcinoma. Pyrosequencing was compared with HRM followed by Sanger sequencing for EGFR and KRAS mutations in specimens obtained by EUS and EBUS. Pyrosequencing on cytological blocks especially older tumor blocks is feasible. The diagnostic performance of both tests was good. HRM failed in a few samples in which the DNA was degraded as a result of longer sample storage times.

Diagnostic success rates

The high diagnostic success rates of both pyrosequencing (97%) and HRM (93%) in this study confirm that cytological aspirates from EUS and EBUS are suitable for molecular analysis. In a few samples we were unable to yield DNA with sufficient quality for HRM and pyrosequencing. After DNA amplification, HRM failed in a few more samples as well. In contrast to HRM analysis, pyrosequencing was successful in all samples indicating a better sensitivity.

EGFR mutation analysis has been performed previously on various kinds of cytologic samples like pleural effusion cell blocks, percutaneous aspirates[8, 15, 33], pericardial effusion and BAL[9] and also endobronchial [8-10, 12, 14-16, 19, 33, 34] and endoscopic ultrasound guided aspirates[14, 16, 33]. Reported success rates of mutation analysis on EUS-guided or EBUS-guided fine needle aspirates ranged from 72-99% in previous studies[8, 9, 11, 12, 14, 16, 17, 20]

A comparison of the diagnostic performance of pyrosequencing with other sequencing techniques is difficult. In contrast to our study, samples in previous studies were often selected on estimated tumor density and most studies were prospective studies and consequently used relatively younger specimen.

Concordance rates

In all but five patients with amplifiable DNA, HRM could be performed. The concordance between pyrosequencing and HRM was 100% for EGFR and 98% for KRAS. Two samples showed wild type *KRAS* using HRM analysis but turned out to be *KRAS* mutated after pyrosequencing. This finding is likely related to the higher test sensitivity.

Two previous reports compared different sequencing methods but none described pyrosequencing. A recent study of 49 cytologic samples of bronchial brushings and pleural effusions compared five different sequencing tests (PCR-Invader®, peptide nucleic acid-locked nucleic acid PCR clamp, direct sequencing, Cycleave™ and Scorpion Amplification Refractory Mutation System (ARMS)®)[18]. Concordance rates between different methods ranged from 93.1-100% (bronchial brushings) and 85-100% (pleural effusions). In a report on 94 patients, Sanger sequencing was less sensitive for detecting *EGFR* mutations than (ARMS)®[10].

Tumor cell density

In this study a significant, although modest correlation was found between the estimated tumor cell density and the allele frequency of different *EGFR* and *KRAS* mutations.

Previous studies used predefined cut off values of tumor cell density to consider samples suitable for molecular analysis[8, 13, 16, 33]. The cut off values ranged from 25%[33] to 70%[13]. In one report, authors claimed that as little as eight tumor cells from paraffin-embedded or fresh specimens obtained after microdissection were considered sufficient for mutation analysis in various cytological specimens[9]. Other studies on molecular analysis of cytological samples described no cut off values at all[10-12, 15, 18]. Estimation of tumor cell percentages is not as relevant as previously stated. Moreover, tumor content is a subjective measure with interobserver variation. Finding a mutation in a sample with very low tumor density is considered a true positive finding. However, the finding of a wildtype in a very low tumor density sample could result in a false negative interpretation of a present mutation. In addition to this technical issue the question remains whether these very low frequency mutations are clinically significant. Are they drivers or bystander mutations?

Sample age

A relationship between sample age and DNA chain lengths after PCR was demonstrated. All HRM failures showed short DNA fragments in the ladder PCR suggesting a relationship between sample age and successful molecular analysis. Nevertheless, we found only a few samples unsuitable for molecular analysis, despite long storage times for up to 3.5 years.

This observation is relevant in case of using archival cytological specimen. Subtyping and staging is frequently performed at once in stage III NSCLC by EUS or EBUS. However, there is no clinical reason to analyze the mutation status in stage III disease since treatment with TKIs is not indicated according to present insights. Most patients with stage III disease however, progress to stage IV during follow up. When adequate material is available for these patients, samples can easily be reprocessed for molecular analysis without new invasive tests.

Pyrosequencing in relation to other assays

The high concordance rate between HRM and pyrosequencing, demonstrates the value of both tests for DNA analysis of EUS and EBUS derived samples. Since previous reports selected samples based on tumor cell percentages and were obtained from different sites with various sampling techniques, comparison with other methods is difficult. In contrast to previous studies, this study used samples derived from archival tissue with suboptimal (fragmented) DNA.

Comparable diagnostic performances for EGFR and KRAS mutation analysis however, were described with COLD-PCR in combination with Sanger sequencing in EBUS samples, enabling the detection of mutation frequencies as low as 5-10%[20]. Tumor percentages in the same range of 5-10% allowed for mutation detection in our study as well although there is some doubt to call a sample a wild type when no

mutations are detected in these samples. With pyrosequencing, analysis in samples with tumor percentages below 5% is feasible and can be used to confirm HRM analysis.
Other features, particularly cost aspects, are important to accomplish the comparison between pyrosequencing and other methods.

Financial considerations
The least expensive method is HRM combined with Sanger sequencing. Pyrosequencing is too expensive to be used as a routine method in daily practice. The financial gap between HRM followed by Sanger sequencing and pyrosequencing is irreconcilable, even when more patient samples are analyzed in one run. More relevant is the comparison of costs between HRM prescreening followed by Sanger sequencing and HRM prescreening followed by pyrosequencing. A 38% increase in expenses was calculated for the latter option.
The difference in costs when Sanger sequencing is replaced by pyrosequencing for samples with an abnormal HRM, is substantial, nevertheless, replacement of Sanger sequencing by pyrosequencing does have considerable advantages (described below) that compensate the surplus of costs to some extend

The position of pyrosequencing in mutation analysis
Sanger sequencing is performed on amplified DNA following HRM analysis. Failure of the HRM, due to poor DNA quality, will consequently result in a failure for Sanger sequencing as well.
Identification of mutations with pyrosequencing, if the HRM melting curve and difference plots show deviations from the wild type curves, could serve as an alternative for Sanger sequencing. An important advantage of such a combined approach is that the molecular analysis is based on two independent techniques allowing for a more confident molecular diagnosis.
The high sensitivity of pyrosequencing enables detection of low-frequency mutations and analysis in samples with a low tumor content or fragmented DNA. Sample characteristics such as tumor cell percentage, older sample age and short DNA fragments in the ladder PCR (for example below 200 bp), could serve as criteria to choose pyrosequencing over Sanger sequencing.

Results of mutation analysis
The *EGFR* mutation incidence was 12% and is somewhat lower when compared to large European series (14-16.6%)[3, 35]. In contrast, the incidence of *KRAS* mutations in this study (42%) is considerably higher compared to a large French study (14%) The difference in mutation incidence is likely the result of patient selection. The French study was performed nationwide, and consisted of stage IV patients. Our study population is derived from a relatively small rural area and includes stage III and IV patients. In a previous study from the Netherlands a comparable incidence for *KRAS* mutations (37%) and an even lower incidence for *EGFR* mutations (7%) has been described[14].

Limitations

Important for the patient is a fast diagnostic track to allow a treatment start as soon as possible. Performing HRM as an initial screening step is a swift method to separate wildtype samples from mutated samples. The disadvantage is that this method requires a few days more when HRM abnormalities have to be determined by sequencing (in this study 65% of patients) in contrast to the use of pyrosequencing as the initial screening method.

CONCLUSION

Mutation analysis in EUS and EBUS guided needle aspirates using pyrosequencing is feasible and showed a high diagnostic success rate. The use of cytological specimens did not lead to analytical difficulties and mutation frequencies were similar to known *EGFR* and *KRAS* mutation frequencies in our population. When comparing pyrosequencing to HRM, a high concordance rate was found. All HRM failures were observed in samples with fragmented DNA associated with longer storage times of the formalin fixed and paraffin embedded cell blocks. Mutation analysis by pyrosequencing enables the use of shorter DNA fragments increasing the yield of molecular analysis on older and less optimal tissue samples.

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e-Table 1
Primers used for multiplex ladder polymerase chain reaction

AF4	Forward	5'-CCGCAGCAAGCAACGAACC-3'
	Reverse	5'-GCTTTCCTCTGGCGGCTCC-3'
PLFZ	Forward	5'-TGCGATGTGGTCATCATGGTG-3'
	Reverse	5'-CGTGTCATTGTCGTCTGAGGC-3'
RAG1	Forward	5'-TGTTGACTCGATCCACCCCA-3'
	Reverse	5'-TGAGCTGCAAGTTTGGCTGAA-3'
TBXAS1	Forward	5'-GCCCGACATTCTGCAAGTCC-3'
	Reverse	5'-GGTGTTGCCGGGAAGGGTT-3'

e-Table 2
EGFR and KRAS HRM primer pairs with the M13 moiety underlined

EGFR exon 18	Forward	5'-GTAAAACGACGGCCAGGACCCTTGTCTCTGTGTTCTTGT-3'	213bp
EGFR exon 18	Reverse	5'-CCACCAGACCATGAGAGGC-3'	
EGFR exon 19	Forward	5'-GTAAAACGACGGCCAGCGTCTTCCTTCTCTCTCTGTCAT-3'	146bp
EGFR exon 19	Reverse	5'-ACACAGCAAAGCAGAAAC-3'	
EGFR exon 20d	Forward	5'-CACCTCCACCGTGCAG*CTC-3'	97bp
EGFR exon 20d	Reverse	5'-GTAAAACGACGGCCAGCAGGTACTGGGAGCCAATA-3'	
EGFR exon 20p	Forward	5'-GTAAAACGACGGCCAGCCACACTGACGTGCCTCT-3'	216bp
EGFR exon 20p	Reverse	5'-AGCTGCGTGATGAGT*TGCA-3'	
EGFR exon 21	Forward	5'-GTAAAACGACGGCCAGTCCCATGATGATCTGTCCCTCACAG-3'	201bp
EGFR exon 21	Reverse	5'-TGCCTCCTTCTGCATGGTATTCTT-3'	
KRAS exon 2	Forward	5'-GTAAAACGACGGCCAGTCACATTTTCATTATTTTTATTATAAGGC-3'	117bp
KRAS exon 2	Reverse	5'-GATTCTGAATTAGCTGTATCGTCAAG-3'	
KRAS WT probe		5'-CTTGCCTACGCCACCAGCTCCAACT[SpcC3]-3'	
KRAS exon 3	Forward	5'-TGTGTTTCTCCCTTCTCAGGA-3'	158bp
KRAS exon 3	Reverse	5'-GTAAAACGACGGCCAGTGGCAAATACACAAAGAAAGC-3'	

Chapter 11

Thoracic masses; from chest radiography and ultrasound guided biopsies to molecular biology.
Review

Submitted

Jos A. Stigt, Harry J. M. Groen

CONTENTS

ABSTRACT

For the analysis of abnormal chest radiographs that are suspicious for a thoracic malignancy, pulmonologist have a large and variable array of diagnostics such as thoracic imaging with computerized tomography (CT), positron emission tomography (PET) or magnetic resonance imaging (MRI), invasive (endoscopic) tests and subsequently pathologic and molecular tests to determine stage and diagnosis of the patient.
Depending on availability of tests and skills, pulmonologists will structure the diagnostic path in close dialogue with the patient in such a way that a minimum number of investigations are required delivering maximum information in the shortest possible time span.
The optimal sequence is first imaging to localise the primary tumor and possible metastases, establishing a diagnosis by histology from the best accessible tumor and staging by (endoscopic) needle aspirations. Although expensive and superfluous for a benign diagnosis, FDG-PET/CT is the optimal starting point when malignancy is suspected. Sampling for pathology should preferably result in a diagnosis and confirmation of disease stage at one time. Histology is preferred for a diagnosis but with adequately provided cytologic material, refined characterization of most primary pulmonary malignancies is possible even up to the level of molecular analysis.
In this review, the optimal diagnostic approach from an abnormal chest radiograph are described and choices and sequences of diagnostic steps are evaluated.

INTRODUCTION

The analysis of thoracic masses found on standard chest radiographs or computerized tomography (CT) is one of the most challenging activities of pulmonology practice. An immediate and adequate diagnostic approach is obliged to shorten the patients' anxious uncertainty about diagnosis and outcome.
Starting off from baseline information, medical history, comorbidity, the assessment of risk for a thoracic malignancy, choices have to be made for further diagnostic analysis while taking into account the wishes of the patient and the consequences for eventual treatment.
The headlines for further evaluation can be divided in the use of different imaging techniques, invasive mainly endoscopic procedures and pathologic and molecular analysis. Important is the timely sequence of different diagnostic modalities. The aim is to obtain maximum information with a minimum number of tests in order to reduce the burden for the patient and to finalize the diagnostic track in the shortest possible time.
In this review we describe the diagnostic procedures to evaluate thoracic masses and evaluate optimalization of integrated diagnostic modalities. Surgical diagnostic procedures are beyond the scope of this review.

IMAGING

Chest radiography

An abnormal standard chest radiography, suspicious for malignancy, is the main reason for a diagnostic referral to a hospital or medical center.
Further imaging of masses or tumors on chest radiography is almost invariably required[1] and has two major purposes: anatomic localisation of the abnormality and its characterization, and determination of extensiveness (staging). For an optimal evaluation, the necessary information is often derived from combinations of imaging modalities often requiring contrast media to delineate mediastinal lymph nodes or to explore the presence of abnormal vessels.

Computerized tomography

Advanced imaging by means of a CT is generally performed as the next step to determine nature, size and exact site of disease[2]. In case of malignancy, local tumor characteristics, hilar and mediastinal metastatic disease as well as distant metastatic disease are visualized and contribute to a clinical TNM classification[3].
There are however serious limitations to CT interpretation. The diagnostic accuracy to determine the T-stage with CT is low[4]. A sensitivity of 63% in distinguishing T3-T4 tumors from T0-T2 tumors and a specificity of 84% were reported [5]. For mediastinal metastatic disease the figures are comparable with a sensitivity and specificity of 51% and 85% respectively[6]. In 5-15% of patients with apparent N0 disease on CT determined by size (shortest diameter < 1 cm), surgical lymph node sampling will reveal node metastasis[7].

Many patients with newly diagnosed non-small cell lung cancer (NSCLC) have unsuspected extrathoracic metastatic disease. Brain, bone, liver, adrenal and soft tissue metastasis were detected with CT in 21-25% of patients with presumed N0 disease[8, 9]. In routine CT evaluation the upper abdomen is therefore included. Although CT provides indispensable information on anatomic details, the addition of metabolic characteristics and pathologic confirmation accomplishes modern thoracic tumor evaluation.

Positron emission tomography

Adding metabolic information to lesions observed with CT imaging, positron emission tomography (PET) helps in distinguishing benign from malignant masses although inflammatory or infectious disease is often metabolically active and malignant disease sometimes not. The metabolic tracer most used is fludeoxyglucose (FDG). FDG-PET is essential in guiding subsequent diagnostic steps or justifying a wait-and-see strategy.

In NSCLC PET is essential for mediastinal staging as well as for detecting distant metastatic disease. The sensitivity and specificity of PET scanning for identifying mediastinal lymph node metastasis are 74% and 85%, respectively[6]. Non-palpable supraclavicular metastasis can be demonstrated by FDG-PET with a diagnostic accuracy of 71% [10]. For detection of unanticipated metastatic disease, FDG-PET is also valuable. In 6.3% to up to 19% of patients with tumors considered resectable, PET identified metastasis and so precluded unnecessary surgery[11-13].

In small cell lung cancer (SCLC), stage migration was described in a median of 13% in several studies after imaging with PET. Not only upstaging, due to improved detection of distant metatatic disease, but also downstaging was observed. Due to the poor quality of these small studies, the role of PET in staging SCLC is however still not clearly established.

Despite the valuable performance characteristics of FDG-PET, there are some limitations. Small lesions (7-10 mm) are usually not detectable as are higher differentiated malignancies like carcinoid and bronchioloalveolar cell carcinomas. For the detection of brain metastasis, FDG-PET has no relevance because smaller lesions cannot be discriminated from the background metabolic uptake of the brains. The positive and negative predictive values for detecting brain metastasis with FDG-PET is very low and magnetic resonance imaging (MRI) or perhaps contrast-enhanced CT perform much better and are standard imaging procedures for suspected brain metastasis.

Integrated PET-CT

The addition of FDG-PET to CT increases the diagnostic accuracy for thoracic malignancies. Integrated PET-CT combines advantages of both imaging modalities and resulted in superior tumor staging and nodal staging compared to CT alone, PET alone or visual correlation of both modalities. And PET-CT facilitates the detection and localisation of distant metastasis[14-16]. Combination with low-dose or diagnostic (contrast-enhanced) CT are possible.

Other nuclear imaging

FDG-PET has become essential in the workup of thoracic masses but apart from quantitative lung perfusion scintigraphy (to estimate postoperative lung function) the role of nuclear imaging is otherwise limited.
The detection of bone metastasis with ^{99m}Tc-labeled phosphonates is sensitive but with the emergence of PET, skeletal scintigraphy diminished. Although the sensitivity of PET to detect skeletal metastases is slightly lower, its specificity of 98% compares favourably with skeletal scintigraphy (61% specificity)[17].
Some neuroendocrine tumors express high levels of somatostatine or dopamine receptors on their cell surface. Especially in carcinoid tumors, often showing less uptake of FDG, radiolabeled somatostatine or dopamine analogues demonstrate the primary tumor and metastasis with high sensitivity[18].

Magnetic resonance imaging

The role of MRI in the evaluation of thoracic masses is limited. Nevertheless, MRI is often applied in the preoperative analysis of superior sulcus tumors because the local assessment of the primary tumor, especially the relationship with cardiovascular structures and neurovascular structures, is superior.
Also for the diagnosis of leptomeningeal and small cerebral metastatic disease, MRI is the best imaging tool. MRI is more sensitive than CT in detecting brain metastasis[19]. Asymptomatic brain metastases are found in 0-10% of patients with NSCLC. Higher incidences of asymptomatic brain metastases are found in stage III patients and MRI is therefore recommended to preclude multimodality treatment[6, 20]. Guidelines on staging SCLC recommend MRI or contrast CT of the brain for all patients[21].

SAMPLING PATHOLOGIC SPECIMEN

Noninvasive diagnostics

With sputum cytology a diagnosis of lung cancer can be established with a high specificity (99%) and without aggravating investigations for the patient. The sensitivity however is low (pooled sensitivity of 66%) and depends highly on tumor location, sputum collection and processing logistics[22] In general it only has a limited role in diagnosing central endobronchial tumors.

Invasive diagnostics

Evaluation of local disease
Almost all thoracic masses require tissue sampling for a definitive diagnosis and only a few diagnoses are made on radiologic appearance alone like bronchogenic or cardiac cysts and hydatid cysts.
Distinguishing benign disease from malignant disease is difficult on CT alone. PET is helpful in determining the need for further diagnostic interventions. When masses are not FDG avide, differential diagnostic considerations narrow and often justify a more observational attitude. In our own diagnostic program for abnormal standard chest

radiographies, 88% of benign diagnoses was based on integrated FDG-PET-CT without pathologic confirmation but with follow up [23].
To analyze a thoracic lesion without evidence of mediastinal or distant metastasis, several techniques are available to obtain tissue specimen for a pathological diagnosis.
For central lesions, bronchoscopy is preferred by many pulmonologists for its diagnostic sensitivity of 89% but for peripheral lesions the sensitivity decreases to 69% and even 33% for lesions smaller than 2 cm.[24]. The performance of bronchoscopy can be increased for these small lesions when sampling is guided by a radial ultrasound (US) probe [25]. Another technique to improve results for sampling peripheral lung lesions is electromagnetic navigation but the impact on diagnostic results still has to be established and weighed against the costs[26].
Centrally located tumors invading or originating from the mediastinum or hilus or situated in close contact with the mediastinum can be diagnosed with bronchoscopy, endoscopic ultrasound (EUS) or endobronchial ultrasound (EBUS) depending on site and preference of the endoscopist. For EUS and EBUS both a diagnostic accuracy of 94% was reported for mediastinal masses of unknown origin and without central endobronchial involvement[27, 28].
Transthoracic needle aspiration (TTNA) or core tissue biopsies (CTB) performed under CT or ultrasound (US) guidance is very helpful to diagnose peripheral and pleural lesions. The sensitivity of CT guided percutaneous biopsies is 92% but the sensitivity depends obviously on size[22].
US guided transthoracic sampling has practical advantages over CT guidance because it is widely available, easy to learn and cheap. The real-time characteristic of US facilitates the biopsy procedure and increases safety. Lesions however must lie in contact with the pleura or originate from the pleura. If these criteria are fulfilled, high diagnostic success rates are possible[29-31].
For mesothelioma, image-guided sampling with either CT or US has a good diagnostic performance (sensitivity of 88%) [32]. Since mesothelioma presents in most cases with pleural effusion, thoracoscopic evaluation is preferred because immediate pleurodesis after evacuation of the effusion is possible. When mesothelioma presents without pleural effusion or with loculated effusions, US guided CTB is a very useful and safe diagnostic alternative as was recently demonstrated in our own series[33].

Evaluation of nodal disease
The analysis of primary thoracic malignancies requires special attention for hilar, mediastinal and supraclavicular regions because lymphogenic spread of primary pulmonary tumors evolve to these nodes and determine the N-stage of disease. Hilar regions are easily accessible with ultrasound guided transbronchial needle aspiration (EBUS-TBNA). The excellent diagnostic characteristics of EBUS provide a diagnosis in most benign or malignant hilar disease sites[34, 35]. Although the finding of hilar metastasis in lung cancer does not preclude surgery, the extend of resection may depend on hilar involvement. Confirmation of hilar involvement during the

preoperative analysis is also important to determine the need for further assessment of upstream lymphogenic mediastinal spread.
The sensitivity of aspirations of hilar lymph nodes (stations 10 and 11) is not different from the sensitivity of aspiring mediastinal lymph nodes as was demonstrated in a large multicenter study[36].
Before US guided devices were developed, blind CT guided transbronchial needle aspiration (TBNA) was used to sample suspected mediastinal lymph nodes. Despite reasonably high accuracy rates in experienced hands and a high range of variation in yield described in reviews, a head to head comparison showed superiority of EBUS[37].
Nowadays the preferred first step in the approach of suspected mediastinal metastatic disease is EBUS-TBNA, endoscopic ultrasound guided fine-needle aspirations (EUS-FNA) or combinations of both. The diagnostic performance of these minimally invasive staging devices is outstanding and pushed surgical staging (mediastinoscopy) to the background in the last decade [38]. In a recent study, mediastinal staging with EUS and EBUS followed by mediastinoscopy (in case of negative endoscopy results) had higher sensitivity than surgical staging alone[39].
Even in patients without visible mediastinal nodes on CT, EUS was able to detect metastasis in 25% of patients with lung cancer[40]. A report on EBUS in patients with a negative mediastinum on PET and CT, demonstrated in 9% of patients with stage I disease mediastinal metastasis[40][41]. The combination of both EBUS and EUS in a CT negative mediastinum was better than EBUS alone and almost significantly better than EUS alone[42].
Supraclavicular lymph nodes are frequently involved in thoracic malignancies and are important for prognosis and therapeutic decisions.
After visualization with PET-CT, adequate pathologic sampling by fine needle aspirations (FNA) is possible under US guidance with a diagnostic accuracy of 85%[31].

Evaluation of distant metastatic disease
If imaging shows abnormalities suspect for metastatic disease of a primary thoracic tumor the most efficient way to make a diagnosis and confirm disease stage is to perform needle biopsies. Pleural effusion, soft tissue, destructed bone, axillary, retromandibular, retroauricular, intercostal and skin metastasis can be sampled after palpation, under CT-guidance or under real-time US guidance with high success rates if not surrounded by air or covered by bone[31].
Deeper located lesions eg. in the left adrenal gland, the celiac region, the spleen, the pancreas or left liver lobe can be reached by EUS-FNA effectively[43, 44].
When imaging obviously demonstrates metastatic disease that is hard to reach for pathologic sampling, it is recommended in guidelines to approach easier accessible lesions like the primary tumor or a lymph node to obtain tissue specimen[22].
Nevertheless, clinical situations exist that require surgical procedures such as (wedge) resections to verify metastatic disease like for instance multiple lung lesions or a solitary right adrenal metastasis.

PATHOLOGIC ANALYSIS

A pathologic analysis of tissue samples is the next step in diagnosing thoracic abnormalities such as those suspect for malignancy. For the diagnosis of lung lesions in general, histologic specimen, with their intact morphology, are preferred for pathologic analysis but that is particularly the case in benign and infrequent diagnoses. It was demonstrated that percutaneous tissue core biopsies were relatively more adequate than needle aspirations for a definitive diagnosis of lung lesions in general[45].

Differentiating SCLC from NSCLC

The main part of patients referred for thoracic masses, finally receive a diagnosis of lung cancer[23, 46]. The choice of treatment is dependent on histological subtyping and pathologic results also determine the need for further immunohistochemistry and molecular analysis [47, 48].

To differentiate SCLC from NSCLC, a morphologic examination is usually adequate and can be performed on both histologic and cytologic material. An almost perfect interobserver agreement for distinguishing SCLC from NSCLC was described recently for all specimen types[49].

Subtyping NSCLC

A diagnosis of NSCLC needs further subtyping. Distinguishing adenocarcinoma from squamous cell carcinoma is of major importance for treatment and the number of patients with a final diagnosis of unclassified NSCLC (NSCLC-NOS) should be minimized.

The diagnosis is generally made on small biopsies or cytology. In comparative studies, small biopsies were not superior to cytology in differentiating the subtypes. The combination of both however greatly reduced the number of NSCLC-NOS and so it is recommended to review, if available, both together in order to achieve optimal specification and concordance. A recent guideline did not express a clear preference for the kind of material. Small biopsies are not indisputably superior to certain cytologic preparations since biopsies often contain stromal reaction and aspirates or effusions may contain more tumor cells[50].

When cytology is obtained it is important that cell blocks are prepared in order to enable further immunohistochemical and molecular diagnosics. In all kinds of cytologic samples, a NSCLC subclassification could be made on a combination of morphology and immunocytochemistry in 94% and 100% of cases[51, 52]. Immunohistochemistry should be performed tactfully with a small but sensitive panel of markers, in order to preserve the limited resources of material for additional molecular testing[53].

The major factors impeding subtyping were poor differentiation, low specimen cellularity and squamous histology but it is unclear if larger samples could solve this problem[52].

Tumor heterogeneity

Another phenomenon disturbing interpretation of pathology results is tumor heterogeneity. For lung cancer this heterogeneity is evident for biomarker expression as well as occurrence of mutations and is described between primary tumor and metastasis or within tumor sites[54]. It is unknown how and to what extend sampling mode (biopy versus aspiration) or sampling site and number could influence the results.

Diagnoses other than lung cancer

For benign disease, the diagnostic results of cytology, as obtained with EBUS, were significantly lower when compared to malignant disease[55]. For rare thoracic diseases the diagnostic performances of biopsy devices and the advantage of histology over cytology are unknown. For more common diseases such as sarcoidosis and malignant lymphoma, several reports have been published. Sarcoidosis can be diagnosed very well with fine needle aspirates. With EBUS, performed in patients with sarcoidosis, a diagnostic yield of 84% was described in a prospective study[56]. And after a negative bronchoscopy, with EUS a diagnostic sensitivity of 71 was found in a large multicenter study[57].
Malignant lymphoma, situated in the mediastinum, can be diagnosed with EUS when the aspirates are analyzed with additional flow cytometry[58]. In this study however, in 3 out of 11 lymphomas, exact subtyping in histology was still required. In a study of 10 patients with lymphoma diagnosed with EUS (with flow cytometry), the diagnostic sensitivity was 74%[59]. The diagnostic sensitivity of EBUS in a study in 21 patients with lymphoma was 57%[60]. Diagnosing lymphoma with EBUS was found more difficult than lung cancer in a study describing the diagnostic performance of EBUS[55].

MOLECULAR ANALYSIS

Currently relevant tests

In patients with NSCLC, certain mutations in the epidermal growth factor receptor (EGFR) gene predict good clinical response to treatment with tyrosine kinase inhibitors (TKI's). For stage IV patients, molecular analysis to detect EGFR mutations is now an essential part of the routine workup. As these mutations are almost invariably found in samples with non-squamous cell carcinoma, patients can be selected for further molecular analysis based on subtyping results.
Another frequently encountered mutation in adenocarcinomas of the lung is V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS). KRAS is also involved in signalling pathways downstream the EGFR but its clinical significance is not completely clear. DNA mutations or amplifications that have predictive value can be used as molecular diagnostic tool. The presence of a translocation of microtubule associated protein-like 4 with anaplastic lymphoma kinase genes (EML-ALK) leads to ALK protein production that can be blocked with specific ALK inhibitors (crizotinib). BRAF mutations are sensitive for BRAF inhibitors such as vemurafinib or dabrafenib.

This list is increasing quickly and future will tell whether this new targeted and personalized approach will change treatment of lung cancer fundamentally.

Sample characteristics

Recommendations on tissue and test characteristics for EGFR mutation analysis were recently published in a European consensus report[61]. The authors prefer the use of histology but mention the possibility of using cytology for mutation analysis. In large clinical studies comparing chemotherapy with TKI as first line treatment, mutation analysis was almost invariably performed on tissue samples.
Other studies demonstrated however the feasibility of molecular analysis in cytologic specimen of NSCLC for EGFR, KRAS as well as EML-ALK[62].
EGFR mutation analysis has been performed on various kinds of cytologic samples like pleural effusion cell blocks, percutaneous ultrasound guided and CT guided aspirates[52, 62-64], pericardial effusion and BAL[62, 65] endobronchial [52, 63-70] and endoscopic ultrasound guided aspirates[52, 68, 69]. The suitability to perform EGFR mutation analysis in cytologic specimen derived with EBUS-TBNA ranged from 72% to 99%[71].
KRAS detection was also performed in a variety of cytologic specimen like TBNA obtained with EBUS or percutaneous FNA under CT or US guidance and endobronchial brushes and washings[52, 52, 63, 67-69].
Fusions of the ALK and EML4 genes are demonstrated with fluorescent in situ hybridisation (FISH). This technique was also applied on cytologic specimen but only described for EBUS sofar[72].

Tumor heterogeneity

Important in the discussion on sample features is the presence of enough tumor cells in either cytological or histological specimen that deliver high-quality DNA. It is clear that molecular analysis can be performed in specimen with low tumor cell content but still there are some hesitations concerning the representation of FNA specimen for the entire tumor[73]. Tumor heterogeneity is a phenomenon that might influence the results of molecular analysis especially when the diagnosis is made on very small specimen. Low frequency mutations sometimes responsible for resistance to treatment after exposure to specific targeted inhibitors can only be found in samples that contain large number of tumor cells.
Even more confusing is the fact that discordance in mutations or phenotypic expression is not only found within a tumor site but also between between primary tumor and metastasis[54].
These issues are not fully understood and clear recommendations for the clinician in this respect are not available.

PLANNING OF DIAGNOSTICS

Planning and timing of diagnostics is important to prevent unnecessary tests and investigations and to shorten patients waiting time and uncertainty. Although the

oncologic significance of delay in obtaining a diagnosis is unclear, recommendations on timelines are described in guidelines[74].

Before start of analysis

An estimation of comorbidity and an evaluation of the patients' wishes and expectations are essential to determine whether diagnostics should be applied. It is obvious that a diagnostic program is only sensible when results have therapeutic consequences.

Diagnostic programs

Diagnostic programs for abnormal standard chest radiographs have been described. Benign disease was found in 16-35% of patients with these diagnostic programs[23, 46, 75]

Standard bronchoscopy after CT or PET-CT was used as invasive diagnostic modality in 3 studies[46, 75, 76]. We described our own diagnostic program of invasive diagnostics performed on the same day as the PET-CT[23]. This study is distinctive from other programs in the immediate choice from bronchoscopy, US guided percutaneous biopsies, EUS and EBUS depending on the imaging results. Ideally a pathologic diagnosis and confirmation of disease stage were made at once. In 40% of patients with a malignancy, bronchoscopy was not necessary to perform. The design of our program resulted in a shorter time from start of the analysis to start therapy in patients with malignancies (14.5 versus 26 days) when compared to a program with standard bronchoscopy after PET-CT[46].

The difference is obviously caused by the need for additional staging investigations when only bronchoscopy is performed.

Sequence of diagnostics

Two flowcharts showing the possible diagnostic tracts for patients suspected of having a thoracic malignancy are superimposed in the figure. It is clear that a CT of the chest and upper abdomen as initial advanced imaging modality is adequate in case of a benign diagnosis. When a non-primary pulmonary malignancy is found, also further imaging is usually not necessary.

A CT is also sufficient when stage IV disease is demonstrated and verified by pathologic analysis. However, when stage III disease is demonstrated with CT, a cerebral MRI as well as a FDG-PET scan should be performed for accurate staging (to exclude distant metastases) thereby precluding unnecessary treatments. After FDG-PET-CT, only a cerebral MRI should follow to exclude asymptomatic brain metastasis.

For stages I and II NSCLC applies the same. After CT, further staging with FDG-PET is advised because asymptomatic metastasis will be detected in a substantial amount of patients. When SCLC is diagnosed, standard staging with bone scintigraphy and cerebral MRI or, probably more accurate, staging with FDG-PET and cerebral MRI is warranted for stages I-III.

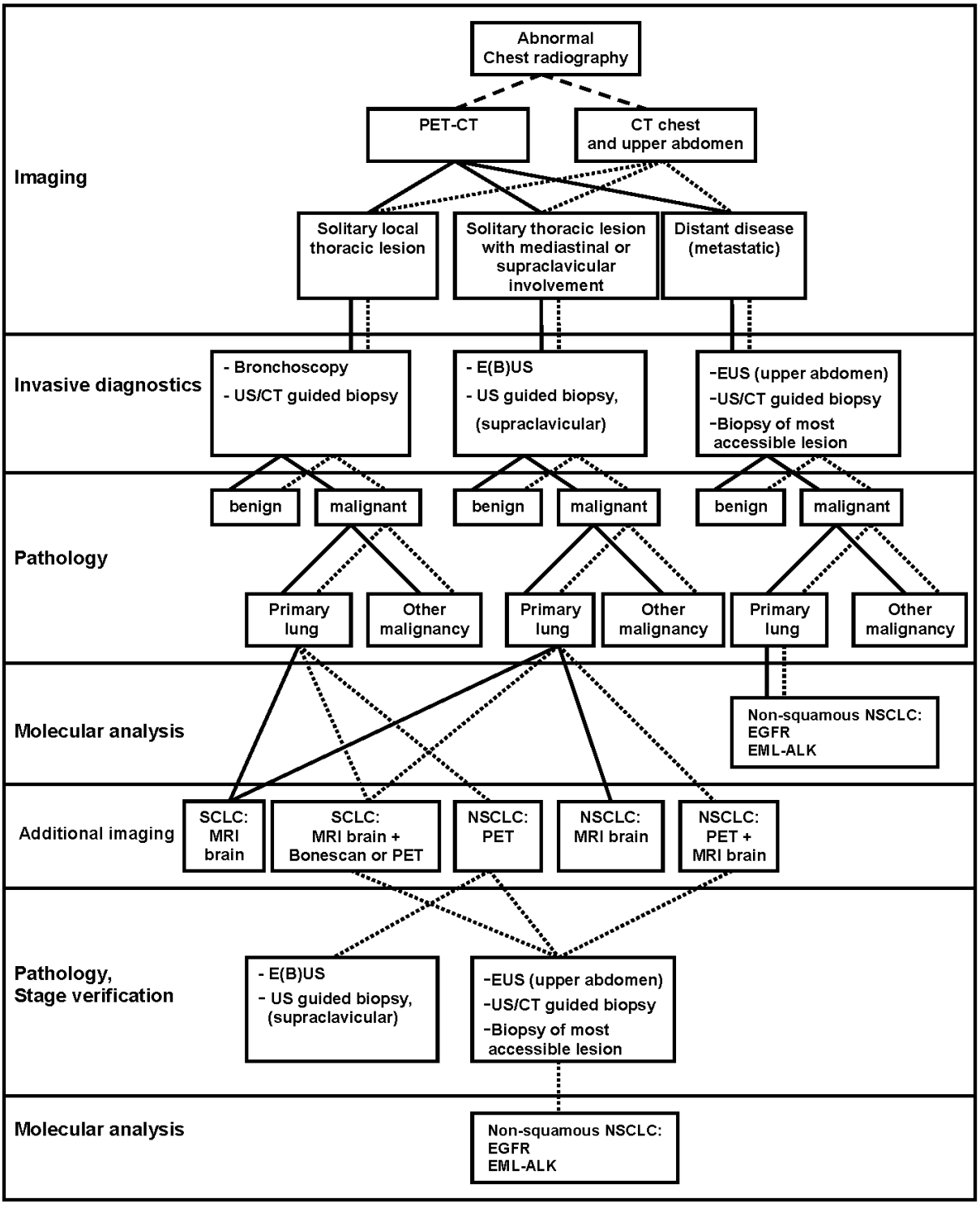

Figure. Flowchart showing the diagnostic tracts of abnormal standard chest radiographs (suspicious for malignancy) starting initially with an integrated positron emission tomography (solid lines) or starting initially with a computerized tomography of thorax and upper abdomen (dotted lines).

PET-CT, integrated positron emission tomography and computerized tomography; CT, computerized tomography; PET, positron emission tomography; US, ultrasound; E(B)US, endoscopic ultrasound and /or endobronchial ultrasound; EUS, endoscopic ultrasound; NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; EML-ALK, microtubule associated protein-like 4 with anaplastic lymphoma kinase gene reallocation; SCLC small cell lung cancer; MRI, magnetic resonance imaging

Additional imaging takes more time in diagnosing patients, for example when new findings appear that need again pathological verification and when non-squamous cell carcinoma is upstaged to IV requiring further molecular analysis. So, apart from a delay in time, patients often have to undergo additional invasive tests that in fact overrule the initial invasive tests. This illustrates the complexity of the lung cancer diagnostic roads.

PET-CT after abnormal chest radiography

From the point of view of diagnostic speed and convenience for the patient, initial imaging with FDG-PET-CT is ideal. The main disadvantage of this diagnostic strategy is that a relatively large number of patients with a benign diagnosis will have this expensive kind of imaging. Two studies on diagnostic tracts with an initial PET-CT, reported 20% and 26% of benign diagnoses[23, 46]. These numbers seem high but when only a CT would have been made, additional FDG-PET scanning would probably have followed in some of these cases to facilitate clinical decision-making. The FDG-PET results also helped in determining the need for invasive diagnostics in many patients as demonstrated by the high rates of benign diagnoses without pathological verification. In both studies describing a diagnostic strategy with initial PET-CT, almost all benign diagnoses were not pathologically confirmed but based on imaging and follow up results[23, 46]. In one study no abnormalities at all were found in 1% of patients[23].

CONCLUSION

The contemporary analysis of thoracic masses stretches, at the base, from abnormal chest radiograph to eventually DNA analysis on tumor tissue at the far end. In between, the pulmonologist has to make choices from an array of many diagnostic options.

A general principle in the sequence of analysis is first imaging and than invasive diagnostics. (There is an exception for cerebral MRI in this context since invasive cerebral diagnostics are hardly performed.) This principle precludes extra (particularly stage verifying) tests.

A general principle in the kind of diagnostic tests is to choose a biopsy mode that enables a diagnosis and verifies disease stage (in case of malignancy) at one time. Histology is preferred (especially for benign diagnoses) but for malignancy, cytologic specimen, provided they are correctly prepared, will do very well even for more advanced processing.

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Chapter 12

SUMMARY AND FUTURE PERSPECTIVES

SUMMARY

Diagnosing thoracic masses is a challenge for the pulmonologist. The diagnostic approaches to evaluate patients are diverse and cover the fields of imaging, interventional pulmonology and pathology, including molecular biologics, to come to a final diagnosis. Exploring and refining these diagnostic applications and fine-tuning of all diagnostic modalities, in order to optimize patient care, is an important task for professionals.
In this thesis, the introduction of some of modalities and a critical appraisal of the integration of several tests is described. Moreover, practical problems in diagnostic approaches have been studied to come to a better patient care, with case reports and explorative studies.

In chapter 2, the results of restaging patients with stage III non-small cell lung cancer (NSCLC) after induction treatment (mostly concurrent chemoradiotherapy) are described. We compared endoscopic ultrasound guided fine-needle aspirations (EUS-FNA) with positron emission tomography (PET).
In 15/28 patients, repeated EUS-FNA in previously tumor-positive mediastinal lymph nodes, showed no tumor cells anymore and only one case was inconclusive. Of the 15 down-staged patients, 11 were confirmed with surgery and 1 showed persistent malignant disease. The 3 other patients had no mediastinal verification because 2 were not operated and 1 had no lymph node dissection. Follow up with imaging was used to assess their fate. There was concordance between pathologic results and PET in 17/27 patients. We concluded that EUS-FNA predicted the absence of nodal metastasis reliably (with an accuracy of 92.3%) and was superior to PET in this respect. Furthermore we concluded that EUS-FNA was safe although many patients suffered from radiation esophagitis.
The relevance of downstaging was illustrated previously by several studies showing that mediastinal downstaging is one of the strongest prognostic factors. The meaning of downstaging for eventual subsequent surgery however, is less clear. The most important question in this particular field is still not solved. We do not know whether surgery should be performed in all downstaged patients after concurrent chemoradiotherapy. The role of further patient selection should be investigated. Has additional surgery a positive effect on survival or is the induction treatment with chemoradiation sufficient in these patients? The observation that vital tumor tissue is still present in resected material of many patients is an argument favouring surgery but the frequent appearance of metastatic disease during the course of many patients is an argument against surgery.
A study to answer this question by randomizing downstaged patients between adjuvant surgery versus a wait and see strategy is never performed.
A second crucial point to address is the question of vitality of tumor cells after treatment. When a pathologist recognizes vital tumor cells in restaging samples, is the presence of these cells prognostic? The same question applies for resected tumor. It could be helpful if reliable biomarkers for cell death were developed.

The diagnostic properties of EUS-FNA are further underscored in chapter 3. Two patients with relatively mild symptoms were referred for EUS-FNA in masses of the posterior mediastinum with involvement of the spine. In both patients EUS-FNA demonstrated infectious spondylodiscitis with staphylococcus aureus. This diagnosis was easily obtained without the need for an orthopaedic diagnostic intervention. Both cases illustrated that thoracic spinal pathology, situated at the frontal side, is not difficult to reach from the lumen of the esophagus. Furthermore do these cases emphasize the importance of ”looking over each others fence” in medicine.
How to increase mutual awareness of techniques and skills of different specialists, acting in adjacent fields is however the question. The key for this is discussing patients in regular multidisciplinary meetings in a hospital or on a regional scale and cooperative publications in literature for a wider scale.

The potential hazard of EUS is illustrated in a case report in chapter 4. A patient with NSCLC, on treatment with bevacizumab, is described who developed a fistula between a mediastinal metastasis and the esophagus after performing EUS-FNA. Although EUS is generally very safe, performing EUS-FNA in patients on treatment with bevacizumab is not recommended.
A disturbance of wound-healing is a known side-effect of bevacizumab but the question remains whether fistula formation in the described patient would not have occurred without bevacizumab. Introduction of micro-organisms from the esophagus into necrotizing tissue might have been sufficient to cause the infection and subsequent fistula formation.

The diagnostic results of ultrasound (US) guided aspirations and biopsies were studied in a population suspected for lung cancer and described in chapter 5. All lesions that were sampled were detected with integrated positron emission tomography and computed tomography (PET-CT). PET-CT was not previously described as the selecting imaging modality for studies on US guided biopsies in pulmonary oncology. But as PET and CT belong to the routine work up of thoracic masses nowadays, the relevancy of US guided sampling is obvious.
A total of 127 patients underwent US guided sampling. The biopsy sites were subdivided into local thoracic, supraclavicular and distant metastatic for staging reasons.
Malignancy, benign disease and inadequate samples were found in 79%, 14%, and 7% of lesions, respectively. The disease stage was confirmed by the aspiration or biopsy in 55% of patients with a malignancy. More than half the lesions were impalpable on physical examination.
It was concluded that US guided biopsies and aspirations based on PET-CT imaging have excellent diagnostic performances in patients suspected for thoracic malignancy.

The accuracy of tissue core biopsies (TCB) obtained under US guidance for patients with mesothelioma without pleural effusion or with only loculated pleural effusion is described in chapter 6. Some mesotheliomas present primarily without pleural

effusion but, more often, it happens that the effusion is already evacuated before a final diagnosis is established. A diagnostic thoracoscopy with biopsies is hard to perform in these patients. Percutaneous US guided tissue sampling is then the least invasive method as diagnostic surgery is the alternative.
From the pathology database of the Isala Clinics, 56 patients with mesothelioma were traced. Twenty of these patients presented without or with locular effusion of which 14 patients were diagnosed with TCB. The diagnostic accuracy was 80% with only one patient with mild haemoptysis as complication. Therefore it was concluded that US guided TCB is worthwhile in this subcategory of patients avoiding more invasive procedures or surgery.

Chapter 7 describes the radiologic and pathologic characteristics of mediastinal lymphadenopathy, detected incidentally on CT scans made for purposes other than oncologic evaluation.
The relevance of this study becomes evident in a time where much more imaging is performed for all kinds of indications such as non-oncological indications like pulmonary embolism or coronary angiography. Physicians faced with these “incidentalomas” often want to exclude malignancy (especially malignant lymphoma) and refer patients for EUS or endobronchial ultrasound (EBUS) to obtain a pathological diagnosis.
This study was performed in 83 patients from 8 different hospitals. The mediastinal lymph nodes were characterized by multiplicity (with a median number of enlarged nodes of 7), small size (range between 6 and 14 mm) and hilar node involvement (in 77%). Needle aspirations showed adequate lymphocellular samples in 66%, well-formed granulomas compatible with sarcoidosis in 22% and were undeterminate in 8% of patients. A FDG-PET was available in 29 patients and showed elevated metabolic activity in 87%. During follow up, 2 patients developed lung cancer, 2 years after mediastinal analysis.
Because the predictive value for malignancy in these mediastinal incidentalomas was very low, a restrictive policy towards invasive diagnostics is warranted and PET has no added value in this study.
The question is whether significant diagnoses will be missed without pathologic sampling. Our study does not support this neither supports routine follow up with CTs. There is no evidence for a particular approach. Perhaps scheduling a repeat CT after a few months is a practical way in outpatients clinics.

A diagnostic program for patients with an abnormal standard radiograph, suspected for lung cancer is described in chapter 8. All patients followed a standardized scheme with an initial PET-CT in the morning and invasive diagnostic tests in the afternoon. The invasive tests were bronchoscopy, EUS, EBUS and US guided biopsies or aspirations. The invasive tests chosen should ideally provide a tissue diagnosis and confirm disease stage in case of malignancy. The aim was to improve diagnostic efficiency by shortening the diagnostic track and by reducing the number of invasive tests per patient.

A total of 297 patients were analyzed resulting in a diagnosis of malignancy in 72%, a benign diagnosis in 26% and no abnormalities at all in 1%.
For 85% of patients with a malignancy, the diagnostic one-day program was sufficient to provide a diagnosis (including disease stage confirmation) although definitive pathologic results had to be awaited and was communicated with the patient at the next outpatient visit. The diagnosis was made with 1 invasive test in 53%, 2 or more tests were performed in 33% and diagnostic surgery was necessary in 8% of patients with malignancy. Bronchoscopy was performed in 59% of patient with a malignant diagnosis.
The median time from the start of analysis to the time that the final results and therapy were discussed with the patient, was 7 days (range,0-50).
It was concluded that the program provided a diagnosis very efficiently with a very short waiting time and without the need of bronchoscopy in a substantial part of patients.
It is clear that speed is important for the patients' mind but less important from an oncologic point of view. This must we weighed against the costs.
A substantial part of our cohort turned out to have benign disease. Although FDG-PET is not clearly indicated in this group, pathologic diagnoses lacked in most of these patients and FDG-PET was essential in excluding a range of diagnoses. On the other hand there was an overshoot of PET scans in patients with a malignancy, with indisputable stage IV disease proven by the CT scan.
Furthermore, from a practical perspective, the one-day program decreases the efficiency of the invasive diagnostic ward as it is unknown what kind of further diagnostics will follow; timetable planning is difficult and defensive.

In chapter 9 we looked into the problem of histological versus cytological samples to obtain adequate tumor cells for molecular tests. Histologic samples obtained with EUS-TCB were compared with cytologic samples obtained with EUS-FNA. Mediastinal or left adrenal masses of at least 20 mm in puncture direction were sampled subsequently during one session with both devices (4 needle passes each time in different directions). The samples were assessed independently by pathologists. In case of non-squamous cell carcinomas, further molecular analysis for epidermal growth factor (EGFR) and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations was performed.
The diagnostic performance of EUS-FNA was superior to EUS-TCB with sensitivities of 92% respectively 69% ($p<0.00018$). The sensitivity of both combined was 98%. TCB often contained insufficient material (or no material at all) for molecular analysis resulting in a diagnostic success rate of 46%. This contrasted with the high diagnostic success rate of 95% for mutation analysis in FNA. It was concluded that EUS-TCB had no added value over EUS-FNA in pathologic and molecular analysis of hilar, mediastinal and left adrenal masses.

DNA pyrosequencing is compared with High Resolution Melting (HRM) for EGFR and KRAS mutations in chapter 10. The tests were performed on EUS and EBUS derived samples (from adenocarcinomas) that were stored as formalin fixed paraffin-

embedded tissue blocks. HRM screens proceeding Sanger sequencing was the routine procedure for mutation analysis. Pyrosequencing is very sensitive and requires only small amounts of tumor DNA.
In 126 samples, the analysis success rates for pyrosequencing and HRM were 97% and 93%, respectively. A significant relation between sample age and DNA fragmentation was observed. As all HRM failures had highly fragmented DNA, this suggests that age might have played a role in the failing HRM analysis.
Many previous studies selected cytologic samples for mutation analysis on estimated tumor content. In our study there was only a weak correlation between estimated tumor content and true content as measured with pyrosequencing.
It was concluded that mutation analysis can be performed very effectively on EUS-FNA and EBUS-TBNA samples, with both HRM (followed by Sanger sequencing) and pyrosequencing. Pyrosequencing is influenced less by inferior DNA quality, it can be performed on short DNA fragments, allowing successful molecular analysis on older and less optimal tissue samples. Initial screening with HRM followed by pyrosequencing in case of abnormalities in a low number of tumor cells or in case of HRM failure is proposed for routine clinical practice.
An important issue in this context is the question whether small tissue samples represent the tumor adequately.Tumors are heterogenous with respect to genotype and phenotype and discordance in EGFR mutation analysis between primary tumor and metastasis or within tumor sites has been demonstrated previously[1]. Because aspirations contain limited amounts of tumor cells, the question raises whether cytological samples represent the tumor sufficiently. US guided aspiration however, offers the opportunity to sample lesions in various directions, in longitudinal and transversal planes and at various depths during one session[2]. Endobronchial biopsies, most often used for a diagnosis of lung cancer, do share the same problem of tissue representation as they are obtained from a limited part of the tumor (repeated biopsies from the margins of a lesion). As heterogeneity is also described between primary tumors and metastases it is debatable which tumor site is preferred for a diagnosis. The primary tumor site, as original site of metastatic disease or the metastasis that defines disease stage and is a representative for tumor migration and homing.

One of the most challenging acivities for pulmonologists is the analysis of patients with abnormalities suspected for malignancy. A wide range of diagnostics is at their disposal. Those can be divided into imaging, interventional pulmonology and pathology. From a simple chest radiograph to a PET-CT up to refined DNA sequencing there is a lot of decision-making to do before a final diagnosis and disease stage are definite.
Chapter 11 gives an overview of all relevant diagnostic modalities and a discussion about their mutual relationships and integration. While planning a diagnostic track, a few principles are important to keep in mind.
To avoid unnecessary invasive tests (especially stage verifying tests), it is important to plan imaging before invasive diagnostics. Furthermore, it is preferable to choose a

biopsy mode that enables pathologic classification and stage verification (in case of malignancy) at one time.
Although histology is preferred (especially for benign diseases and poorly differentiated malignancies), cytologic samples, if processed properly, will generally provide a diagnosis and with sufficient tumor cells, an analysis of mutational aberrations.

FUTURE PERSPECTIVES

Imaging

At the moment, screening for lung cancer in patients with elevated risk profiles is a hot topic in imaging. A relative reduction in mortality from lung cancer in high-risk patients with low-dose CT screening of 20% was described recently[3]. A problem of screening is the high rate of false positive results requiring analysis or follow up. Furthermore, small nodules (often of subcentimeter size) are frequently encountered in daily practice as coincidental findings on CT scans made for all kinds of indications.
All these small abnormalities require advanced analysis. Improvement of imaging technique to qualify them reliably is a major effort for equipment manufacturers. New PET tracer may be more specific than FDG. Also technological improvements in PET will bring a better resolution. The question is, whether all these novelties will improve diagnostic pathways.
Algorithms integrating combinations of non-invasive diagnostics such as biomarkers have to be validated in the future to differentiate early stage malignant disease from benign findings.
A promising innovation to combine with state of the art CT imaging in the analysis of small abnormalities is exhaled breath analysis[4]. Though not yet sensitive enough, it is expected that in the future, breath analysis might play an important role in decision making for very small lesions. Other biomarkers such as circulating tumor cells and circulating DNA in serum seem promising.

Interventional pulmonology

The incredible diagnostic shift that endoscopic and endobronchial ultrasound has made in pulmonology is hard to repeat because we are able now to diagnose formerly inaccessible anatomic areas.
Other non-surgical interventional modalities for the diagnosis of thoracic masses are not evident. Minor steps are made in improving the quality and user comfort of needles for EUS, EBUS and percutaneous applications.
Reports on EUS guided therapy in gastroenterology (particularly in pancreatic disease) with a flexible bipolary probe, combining radiofrequency ablation with cryothermal cooling are promising also for thoracic approaches[5]. In the future it is foreseen that EUS and EBUS will be applied for therapeutic purposes as well in pulmonology.

For pulmonologists, percutaneous US has gained ground for the analysis of pleural effusions but still with restraints. Only a small step further is the use of US to obtain more accurately samples for pathology, a field that was previously reserved for interventional radiologists. Similar to other disciplines, like cardiology, urology and gynaecology, percutaneous US should become an integral part of the pulmonology practice as well.
Although it is reasonable that EUS and EBUS are performed by dedicated pulmonologists in specialized centers due to high equipment costs and limited numbers of patients, for percutaneous US the situation is different.
Percutaneous US is cheap, applicable on a daily base and easy to learn. Training in percutaneous US skills, now limited to a few centers, should be offered to every pulmonologist during their education. More diagnostics in one hand has great advantages for the patient because it increases diagnostic speed and efficiency.

Even though diagnostic surgery is not quite classifiable as interventional pulmonology, it should be mentioned in this context. In recent years, important developments in diagnostic surgery have been established. Minimal invasive surgery evolved and lowered the threshold to consult the surgeon for mediastinal and upper abdominal exploration. Initially, video-assisted thoracoscopic surgery (VATS) for diagnosing lung lesions and otherwise inaccessible hilar or mediastinal lesions was established. Gradually, robotic control is introduced in VATS, enabling even more precise handling of instruments and enabling access to more difficult sites in the thorax.

Pathology and molecular biology
The developments in this field are of the most exciting kind.
New predictive markers for lung cancer are detected and validated with high speed. Immunohistochemistry, fluorescent in situ hybridization and DNA sequencing are all tests that are performed on patient material. However, more tests have to be performed on samples requiring more tumor cells. To counteract this development, next-generation sequencing (NGS) on DNA and RNA from paraffine embedded specimen provide one kind of test that needs only limited amounts of DNA and RNA. This approach is valuable when mutations, focal amplifications, copy number aberrations and certain fusion proteins are predictive for the efficacy of targeted therapeutics. The success of this approach depends partly on the willingness of pulmonologists to obtain larger tumor samples to detect low-frequency mutations. Whole genome sequencing equipment is commercially available that is able to unravel all abnormalities in cancer genes in small amounts of tumor[6]. The clinical meaning of most abnormalities however, is not yet understood.
Nevertheless it is expected that knowledge of oncologic pathways will increase quickly with NGS, resulting in the discovery of more molecular targets and resistance mechanisms. New agents or combinations of agents and more insight in benefits and harms of certain treatments will improve personalized therapy.

The pulmonologist is constantly moving in a field with changing demands of the pathologist, depending on technical developments, at one side and changing understanding of clinical relevancy at the other side.

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Chapter 12

NEDERLANDSE SAMENVATTING

De diagnostiek naar tumoren in de thorax is een uitdaging voor de longarts. De mogelijke benaderingswijzen om tot een uiteindelijke diagnose te komen zijn zeer divers en bestrijken de afbeeldende diagnostiek, de interventie longgeneeskunde en de pathologie inclusief de moleculaire biologie. Het verkennen en verfijnen van deze diagnostische toepassingen en het onderling afstemmen van diagnostische modaliteiten, met verbetering van de patiëntenzorg als doel, behoort tot de taken van de longarts.
In dit proefschrift worden toepassingen van enkele van deze diagnostische modaliteiten geïntroduceerd en wordt de integratie van verschillende tests kritisch geëvalueerd. Verder wordt onderzoek beschreven naar praktische problemen in de diagnostiek, door middel van case-reports en exploratieve studies.

In hoofdstuk 2, worden de resultaten beschreven van restadiëringsonderzoek bij patiënten met stadium III niet-kleincellig longcarcinoom (NSCLC) na inductiebehandeling (meestal gelijktijdige chemoradiotherapie). We vergeleken daarbij resultaten van fijne naald aspiraten, verkregen onder geleide van endoscopische echografie (EUS-FNA), met positron emission tomography (PET). Bij 15/28 patiënten waren geen tumorcellen meer aantoonbaar bij herhaalde EUS-puncties in mediastinale klieren die vóór de inductiebehandeling nog tumor-positief waren en bij slechts 1 patiënt was de uitslag onzeker. Van deze 15 patiënten kon zogenaamde “down-staging” chirurgisch bevestigd worden bij 11 en bleek er toch bij 1 patiënt persisterende tumoractiviteit te bestaan. Chirurgische verificatie vond bij 3 patiënten niet plaats omdat er 2 niet geopereerd werden en omdat bij 1 patiënt geen lymfklierdissectie plaatsvond tijdens operatie. Bij deze 3 patiënten werd het verdere ziektebeloop middels afbeeldende diagnostiek gevolgd.
Er was overeenstemming tussen de pathologische resultaten en de PET resultaten bij 17/27 patiënten. We concludeerden dat EUS-FNA de afwezigheid van kliermetastasen betrouwbaar voorspelde (met een nauwkeurigheid van 92,3%) en in dat opzicht beter presteerde dan PET. Voorts concludeerden we dat EUS-FNA een veilige ingreep was ofschoon veel van de patiënten op het moment van onderzoek een radiatie-oesofagitis hadden.
De relevantie van zogenaamd “downstagen” voor de prognose wordt door meerdere kleine studies geïllustreerd. Mediastinale “downstaging” is één van de sterkste prognostische factoren. De betekenis van “downstaging” voor eventueel aanvullende chirurgische behandeling is echter onduidelijk. De belangrijkste vraag of een operatie toegevoegde waarde heeft bij patiënten waarbij, na gelijktijdige chemoradiotherapie, geen metastasen in het mediastinum meer aantoonbaar zijn, is nog steeds niet beantwoord. De rol van verdergaande patiëntselectie moet onderzocht worden. Heeft aanvullende chirurgie een gunstig effect op de overlevingskansen of is de inductiebehandeling op zich al toereikend ? De observatie dat er bij vele patiënten vaak nog steeds vitaal weefsel in resectiemateriaal aanwezig is, zou een argument

kunnen zijn vóór operatie maar het frequent optreden van afstandsmetastasen tijdens het ziektebeloop pleit weer tegen.
Een studie die deze vraag zou kunnen beantwoorden, door patiënten die “gedownstaged” zijn te randomiseren tussen chirurgie of een afwachtend beleid, werd nooit uitgevoerd.
Een tweede cruciaal punt van discussie is de vraag in hoeverre als vitaal herkenbaar weefsel nog daadwerkelijk vitaal is na voorbehandeling. Als een patholoog nog vitale tumorcellen herkent in restadiëringsmonsters wat heeft dit dan voor een betekenis voor de prognose ? Dezelfde vraag geldt voor resectiemateriaal.
De ontwikkeling van betrouwbare biomarkers voor celdood zouden in deze zeer behulpzaam zijn.

De diagnostische mogelijkheden van EUS-FNA worden verder benadrukt in hoofdstuk 3. Twee patiënten met relatief milde symptomen werden verwezen voor EUS-FNA in verband met massa’s in het achterste mediastinum met aantasting van de wervelkolom. Bij beide patiënten werd middels EUS-FNA een infectieuze spondylodiscitis vastgesteld met staphylococcus aureus als verwekker.
Deze diagnose werd eenvoudig gesteld zonder dat er orthopaedisch chirurgische ingrepen nodig waren. De ziektegevallen tonen aan dat afwijkingen gelocaliseerd aan de voorzijde van de thoracale wervelkolom goed bereikbaar zijn vanuit de slokdarm. Tevens benadrukken deze ziektegevallen het belang in de geneeskunde om als het ware “over de schutting te kijken” bij andere specialismen.
De vraag resteert echter hoe specialisten van belendende disciplines zich beter op de hoogte zouden kunnen stellen van elkaars diagnostische mogelijkheden en vaardigheden. Dit zou bereikt kunnen worden, op kleinere schaal, door multidisciplinaire besprekingen binnen een ziekenhuis of in regionaal verband of, op grotere schaal, door middel van gezamenlijke publicaties in de vakliteratuur.

Een complicatie van EUS wordt geïllustreerd aan de hand van een case report in hoofdstuk 4. Er wordt een patiënt met NSCLC beschreven die, onder behandeling met het middel bevacizumab, een fistel ontwikkelt tussen een mediastinale metastase en de slokdarm, kort nadat EUS-FNA werd uitgevoerd.
Hoewel EUS-FNA over het algemeen als uiterst veilig wordt beschouwd, lijkt het verstandiger om deze ingreep niet te verrichten bij patiënten die behandeld worden met bevacizumab.Een bekende bijwerking van bevacizumab is immers een gestoorde wondgenezing maar het blijft de vraag of fistelvorming, zoals in de beschreven patient, ook niet zou zijn opgetreden zonder dit middel. Door de introductie van microörganismen vanuit de slokdarm naar necrotiserend tumorweefsel zouden op zich al genoeg voorwaarden aanwezig geweest kunnen zijn voor infectie en fistelvorming.

De diagnostische resultaten van echogeleide puncties en biopten, uitgevoerd bij patiënten die verdacht werden van longkanker, worden beschreven in hoofdstuk 5. Alle afwijkingen die werden bemonsterd, waren gedetecteerd met geïntegreerde positron emission tomography en computed tomography (PET-CT).

Het gebruik van de PET-CT om afwijkingen te selecteren voor echogeleide biopten in de longoncologie, was niet eerder beschreven. PET en CT zijn tegenwoordig echter niet weg te denken bij de diagnostiek van thoracale massa's hetgeen de relevantie van echogeleid biopteren van gevonden afwijkingen onderstreept.
Bij een totaal aantal van 127 patiënten werden echogeleide puncties of biopten afgenomen. De te biopteren afwijkingen werden onderverdeeld in lokaal thoracale afwijkingen, supraclaviculaire metastasen en afstandsmetastasen. Deze indeling houdt verband met de implicaties voor tumorstadiëring.
Bij 79% van de monsters werd een maligniteit gevonden, bij 14% een goedaardige ziekte en 7% van de monsters was inadekwaat.
Bij 55% van de mensen bij wie een maligniteit werd gevonden werd het ziektestadium bewezen door de punctie of het biopt. Meer dan de helft van de afwijkingen was niet te vinden bij lichamelijk onderzoek.
De conclusie van het onderzoek was dat de diagnostische resultaten van echogeleide biopten en puncties, van afwijkingen die met PET-CT werden gedetecteerd, uitstekend zijn bij patiënten die verdacht worden van een maligniteit.

De diagnostische nauwkeurigheid van weefselnaaldbiopten verkregen onder echogeleide bij patiënten met een mesothelioom, gekenmerkt door de afwezigheid van pleuravocht of met slechts geloketteerd vocht, wordt beschreven in hoofdstuk 6. Sommige mesotheliomen presenteren zich primair zonder vocht maar de situatie doet zich vaker voor dat het vocht reeds verwijderd is voordat een zekere diagnose werd gesteld. Een thoracoscopie, waarbij tevens pleurabiopten kunnen worden genomen, is in die situaties moeilijk uitvoerbaar. Het afnemen van echogeleide pleurabiopten is dan de minst belastende methode waarmee chirurgisch diagnostische ingrepen kunnen worden voorkomen.
Vanuit de pathologie database van de Isala Klinieken werden 56 patiënten met de diagnose mesothelioom getraceerd. Twintig van deze patiënten presenteerden zich zonder pleuravocht of met slechts weinig, geloketteerd, vocht. Van deze 20 patiënten werd bij 14 de diagnose verkregen door middel van weefselnaaldbiopten. De diagnostische nauwkeurigheid bedroeg 80%. Slechts één patient hoestte na het biopteren een geringe hoeveelheid bloed op als complicatie van het onderzoek.
De conclusie van het onderzoek was dat het zeer de moeite waard is, bij deze categorie van patiënten, om echogeleide weefselnaaldbiopten te nemen zodat, de meer belastende, chirurgische diagnostiek kan worden vermeden.

In hoofdstuk 7 worden de radiologische en pathologische karakteristieken beschreven van mediastinale lymfadenopathie die per toeval werd ontdekt op CT scans die voor allerlei andere dan oncologische indicaties (zoals longemboliën en coronaire angiografie) werden vervaardigd.
De relevantie van deze studie zit hem in het feit dat artsen die geconfronteerd worden met deze zogenaamde "incidentalomen" vaak een maligniteit (met name maligne lymfoom) willen uitsluiten en daarom patiënten verwijzen voor EUS of endobronchiale echografie (EBUS).

Deze studie werd uitgevoerd bij 83 patiënten uit 8 verschillende ziekenhuizen. De mediastinale klieren werden gekenmerkt door hun meervoudigheid (mediane aantal vergrote klieren van 7), kleine omvang (tussen de 6 en 14 mm) en door betrokkenheid van de hilaire klieren (bij 77% van de patiënten). Fijne naaldmonsters toonden adekwate lymfklierpunctaten bij 66% en welgevormde granulomen, passende bij sarcoïdose, bij 22% van de patiënten terwijl in 8% van de gevallen de puncties geen klassificeerbare diagnose opleverden.
Bij 29/83 patiënten was ook een PET-CT beschikbaar en metabole activiteit in de incidentalomen werd hierop bij 87% gezien. Gedurende de follow up periode ontwikkelden 2 patiënten longkanker, beiden 2 jaar na de mediastinale analyse. Aangezien de voorspellende waarde voor maligniteit bij deze mediastinale incidentalomen erg laag was, is een terughoudend beleid ten aanzien van invasieve diagnostiek gerechtvaardigd. De PET-CT heeft bij de analyse van mediastinale incidentalomen geen toegevoegde waarde.
De vraag is of significante diagnoses gemist zullen worden zonder pathologische verificatie. Onze studie ondersteund dit echter niet evenmin als een routinematige follow up met CT scans wordt ondersteund. Er zijn geen duidelijke bewijzen die voor een specifieke follow up pleiten maar wellicht is een herhaald CT onderzoek na enkele maanden voor de reguliere praktijk een praktische benadering.

Een diagnostisch programma voor patiënten met een afwijkende thoraxfoto (verdacht voor longkanker) wordt beschreven in hoofdstuk 8.
Alle patiënten doorliepen een gestandaardiseerd programma bestaande uit een PET-CT in de ochtend gevolgd door invasieve diagnostiek in de middag. Bronchoscopie, EUS, EBUS en echogeleide biopten of puncties stonden hierbij ter beschikking. Het uitgangspunt bij de keuze van het invasieve diagnosticum was dat idealiter zowel een diagnose werd gekregen als het ziektestadium (in geval van maligniteit) werd bevestigd. Het doel van de studie was tweeledig : verbetering van diagnostische efficiëntie enerzijds en terugbrengen van het aantal invasieve testen per patiënt anderzijds.
Een totaal aantal van 297 patiënten werden volgens protocol geanalyseerd hetgeen resulteerde in een diagnose van maligniteit bij 72% en een goedaardige diagnose bij 26% van de gevallen. Bij 1% van de patiënten bleek er in het geheel geen afwijking te bestaan.
Bij 85% van de patiënten met een maligniteit kon de diagnose (inclusief bevestiging van tumorstadium) afgerond worden op de diagnostische dag. Men moet zich daarbij wel realiseren dat de uiteindelijke resultaten van het pathologisch onderzoek op de reguliere manier afgewacht moest worden en derhalve pas kon worden medegedeeld aan de patient tijdens de policontrole die standaard 1 week later gepland stond.
De diagnose werd gesteld met 1 invasieve test bij 53% en met 2 of meer testen bij 33% van de patiënten. Uiteindelijk werd bij 8% van de patiënten alsnog chirurgische diagnostiek toegepast. Bronchoscopie werd uitgevoerd bij 59% van de patiënten bij wie een maligniteit werd vastgesteld.

De mediane tijd, gerekend vanaf de eerste dag van de analyse tot aan het moment waarop de uiteindelijke testresultaten en het voorstel tot therapie met de patiënten werd besproken bedroeg 7 dagen (range 0-50 dagen).
De conclusie van het onderzoek was dat in het kader van dit programma de diagnose op een efficiënte manier kon worden verkregen met een zeer korte wachttijd. Tevens bleek dat bronchoscopie in een substantieel deel van de patiënten niet noodzakelijk was.
Het is duidelijk dat de patiënt over het algemeen zeer tevreden is met een hoge diagnostische snelheid maar het belang vanuit oncologisch perspectief is veel minder helder. Dat moet uiteraard gewogen worden tegen de kosten van overbodige PET scans en personele inzet.
In de categorie van patiënten met een benigne diagnose is een PET-CT niet strict geïndiceerd. Desalniettemin is de informatie verkregen door een PET-scan in deze gevallen vaak uiterst nuttig om een aantal alternatieve diagnoses te kunnen wegstrepen temeer daar een pathologische diagnose in deze groep patiënten vaak ontbrak. Daarnaast had bij een deel van de patiënten met een maligniteit de PET scan nauwelijks toegevoegde waarde als de CT scan al ondubbelzinnige bewijzen voor stadium IV ziekte liet zien.
Vanuit praktisch oogpunt heeft het sneldiagnostiekprogramma wel een ongunstig effect op de efficiëntie van de behandelkamer. Het is immers nog onbekend in de ochtend welke onderzoeken de patiënten in de middag gaan krijgen zodat het plannen van een programma lasting en behoudend is.

In hoofdstuk 9 wordt de vraag beantwoord of histologische naaldbiopten misschien beter zijn dan cytologische naaldbiopten (beiden verkregen door middel van EUS) voor het verrichten van pathologische diagnostiek maar ook voor het uitvoeren van moleculaire diagnostiek.
Hiervoor werden weefselnaaldbiopten uit mediastinale klier- en bijniermetastasen, vergeleken met cytologische punctaten verkregen tijdens dezelfde EUS-sessie uit dezelfde tumorlokalisatie.
De afmeting van de mediastinale of bijnier massa's moest minstens 20 mm bedragen in de prikrichting om gebiopteerd te kunnen worden met de gebruikte histologische naalden. Met beide soorten naalden werd 4 maal bemonsterd waarbij getracht werd de prikrichting binnen de massa te variëren voor optimale representatie van de lesie. De monsters werden onafhankelijk, door verschillende pathologen beoordeeld. Als er sprake was van een niet-plaveiselcelcarcinoom, dan werd aanvullend moleculaire analyse verricht naar epidermal growth factor receptor (EGFR) en V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutaties.
Het bleek dat de diagnostische kwaliteit van de fijne naaldaspiraten beter was dan van de weefselnaaldbiopten met een diagnostische sensitiviteit van 92% voor de aspiraten versus 69% voor de biopten hetgeen significant verschillend was ($P<0.00018$). Wanneer beide modaliteiten gecombineerd worden gebruikt is de sensitiviteit 98%.
De weefselnaaldbiopten bevatten vaak zowel kwalitatief als kwantitatief onvoldoende materiaal (of soms zelfs helemaal geen material) voor moleculaire analyse waardoor

de lage diagnostische success rate van 46% verklaard wordt. Dit in tegenstelling tot de hoge diagnostische success rate van 95% die voor fijne naaldaspiraten aangetoond werd.
De conclusie van het onderzoek was dan ook dat de, middels EUS verkregen, weefselnaaldbiopten geen toegevoegde waarde hadden aan de fijne naaldaspiraten voor de pathologische en moleculaire analyse van hilaire, mediastinale en bijniermetastasen.

Twee strategieën voor EGFR en KRAS mutatie analyse in DNA van patiënten met een adenocarcinoom worden vergeleken in hoofdstuk 10. Van cytologische samples verkregen middels EUS en EBUS en bewaard als formaline gefixeerde paraffine blokjes werd daarvoor zowel een mutatie analyse verricht door middel van de pyrosequencing techniek als met behulp van High Resolution Melting (HRM). Als er een afwijkend uitsmeltpatroon bij HRM werd gevonden volgde aanvullend nog Sanger sequencing voor nadere identificatie van gedetecteerde DNA afwijkingen. De HRM screening met eventueel Sanger sequencing is de standard procedure en werd vergeleken met upfront pyrosequencing, een techniek die gevoeliger is en waarvoor slechts kleine hoeveelheden DNA nodig zijn. Het vermoeden bestond dat pyrosequencing vaker tot een succesvolle DNA analyse zou leiden op materiaal dat vaak al langere tijd in de vriezer bewaard was en dat wellicht ook relatief weinig tumorcellen zou bevatten omdat het cytologische punctaten betreft.
Toch bleken, in deze studie bij 126 monsters, beide analyse methodieken uiterst geschikt gezien de hoge diagnostische success rates (voor pyrosequencing 97% en voor HRM 93%). Er werd een significante relatie gezien tussen de leeftijd van de preparaten en de mate waarin het DNA gefragmenteerd was. Alle mislukte HRM analyses werden gekenmerkt door sterk gefragmenteerd DNA hetgeen suggereert dat de leeftijd van monsters een rol speelde bij de mislukte HRM analyses. Eerder gepubliceerde studies selecteerden cytologisch material op ingeschat tumorgehalte. In onze studie werd maar een zwakke correlatie gevonden tussen geschat tumorgehalte en daadwerkelijk tumorpercentage zoals gemeten met pyrosequencing. De conclusie was dat mutatie analyse uiterst effectief uitgevoerd kan worden op EUS- en EBUS aspiraten zowel door middle van HRM (gevolgd door Sanger sequencing) als door middel van pyrosequencing. Pyrosequencing wordt minder gehinderd door verminderde DNA kwaliteit omdat het op kortere fragmenten kan worden uitgevoerd waardoor succesvolle mutatie analyse kan plaatsvinden op oudere en minder optimale monsters.
Initiële screening met HRM, gevolgd door pyrosequencing als afwijkingen gevonden worden in monsters met een laag tumorgehalte of in het geval dat HRM mislukt, wordt voorgesteld voor routine mutatie analyse in de klinische praktijk.
Een belangrijke vraag in deze context is of kleine weefselmonsters de tumor wel adekwaat vertegenwoordigen. Tumoren zijn heterogeen voor wat betreft hun genotype en fenotype. Er worden daardoor verschillen gevonden bij mutatie analyses tussen de primaire tumor en metastase maar ook binnen één tumorlokalisatie zijn verschillen in mutaties beschreven[1].

Aangezien aspiraten een beperkt aantal tumorcellen bevatten, ontstaat de vraag of cytologische monsters de tumor voldoende representeren. Omdat echogeleide puncties onder direct zicht plaatsvinden is het goed mogelijk om te puncteren in diverse richtingen binnen een tumor zowel in lengterichting als in breedterichting en tot op wisselende diepten zoals gedemonstreerd in bijgevoegde video-link[2]. Endobronchiaal afgenomen biopten, als meest gebruikte materiaal voor de diagnose van longkanker, kennen echter hetzelfde problem. Bronchusbiopten zijn repetitief verkregen uit een zeer beperkt en oppervlakkig deel van de tumor en representeren de tumor derhalve ook maar in beperkte mate.
Omdat heterogeniteit ook beschreven is tussen primaire tumor en metastase is het een punt van discussie welke tumorlokalisatie de voorkeur van sampling heeft. De primaire tumor, als oorsprong van de ziekte, of de metastase die het tumorstadium bepaald en de resultante is van tumormigratie en tumornesteling.

De analyse van patiënten met afwijkende thoraxfoto's, verdacht voor maligniteit, behoort tot de meest uitdagende activiteiten van longartsen. Een breed scala aan diagnostische middelen staat hen ter beschikking. Deze kunnen onderverdeeld worden in afbeeldende diagnostiek, interventie pulmonologie en pathologische diagnostiek.
In het trajekt van simpele longfoto, via een PET-CT tot verfijnde DNA sequencing techniek, moet allerlei besluitvorming plaatsvinden om tot een uiteindelijke diagnose, inclusief tumorstadium, te komen.
In hoofdstuk 11 wordt een overzicht gegeven van alle relevante diagnostische middelen en wordt hun onderlinge samenhang en integratie besproken. Bij het maken van een diagnostisch plan zijn er enkele principes die in gedachten gehouden moeten worden.
Om onnodige invasieve testen te voorkomen (met name stadiumbevestigende testen) is het essentiëel om eerst afbeeldende diagnostiek en dan pas invasieve diagnostiek te plannen. Verder verdient het de voorkeur om een biopsie methode te kiezen die zowel een klassificerende diagnose als een tumorstadium (in geval van maligniteit) oplevert.
Hoewel histologie de voorkeur heeft (vooral bij goedaardige ziektebeelden en slecht gedifferentiëerde maligniteiten), zullen cytologische monsters, mits juist geprepareerd, over het algemeen een diagnose opleveren en, als er voldoende tumorcellen aanwezig zijn, een analyse naar mutaties mogelijk maken.

TOEKOMSTPERSPECTIEF

Afbeeldende diagnostiek

Momenteel is screening voor longkanker bij hoog-risico groepen een hot topic in de afbeeldende diagnostiek. Onlangs werd een afname van de sterfte door longkanker van 20% gezien bij hoog-risico patiënten die met low-dose CT scans gescreend waren[3]. Een probleem bij screening is echter het hoge percentage fout-positieve

CT scans die vervolgens weer nadere analyse of radiologische follow up noodzakelijk maakt.
Verder zien we tegenwoordig zeer vaak kleine afwijkingen (vaak kleiner dan 1 cm.) op CT scans, die om allerlei niet-oncologische redenen worden gemaakt.
Ook deze kleine afwijkingen behoeven weer enigerlei vorm van follow up.
Er is daarom een grote behoefte aan verbeteringen van de afbeeldende technieken, om de kleine afwijkingen betrouwbaarder te kwalificeren. Aldus is er een belangrijke taak weggelegd voor de fabrikanten van deze apparatuur.
Nieuwe PET-tracers worden meer specifiek dan het nu gebruikte FDG. Daarnaast zullen technologische verbeteringen aan de PET apparatuur resulteren in een betere resolutie van beelden. De vraag blijft echter of al deze vernieuwingen ook daadwekelijk een verbetering van het diagnostisch traject opleveren.
Algoritmes die combinaties van niet-invasieve diagnostiek (zoals biomarkers) integreren, moeten worden gevalideerd in de toekomst om het onderscheid te kunnen maken tussen vroeg stadium maligniteit en goedaardige afwijking.
Een veelbelovende innovatie die gecombineerd zou kunnen worden met state of the art CT onderzoek bij de analyse van zeer kleine afwijkingen, is de analyse van uitademingslucht[4]. Hoewel deze techniek nu nog niet gevoelig genoeg is, valt in de toekomst te verwachten dat uitademingsluchtanalyse een belangrijke rol gaat spelen bij besliskundige aspecten van zeer kleine afwijkingen. Andere biomarkers, zoals circulerende tumorcellen en circulerend DNA in het serum lijkt ook veelbelovend in dit verband.

Interventie pulmonology
De enorme diagnostische revolutie die endoscopische en endobronchiale echografie te zien heeft gegeven is niet te evenaren. Voorheen anatomisch ontoegankelijke plaatsen in het lichaam zijn nu goed benaderbaar. Nieuwe, niet-chirurgische, interventiediagnostiek voor de analyse van thoracale massa's hebben zich momenteel nog niet aangediend. Kleine verbeteringen worden gerealiseerd in de kwaliteit en het gebruiksgemak van naalden ten behoeve van EUS, EBUS en uitwendige toepassingen.
Wel zijn er prille ontwikkelingen in de therapeutische toepassingen van EUS in de gastro-enterologie (met name bij pancreasaandoeningen) waarbij in een flexibele bipolaire probe zowel radiofrequency ablatie als cryothermale koeling gecombineerd wordt[5]. Mogelijk dat deze techniek in de toekomst ook geëxtrapoleerd kan worden naar EUS en EBUS zoals toegepast in de pulmonologie.
Schoorvoetend zijn longartsen de uitwendige echografie gaan toepassen voor de analyse van pleuravocht. Het is echter maar een kleine stap verder om de echografie als hulpmiddel te gebruiken bij het verkrijgen van materiaal voor pathologische diagnostiek; een terrein dat eerder vrijwel exclusief in handen was van de interventieradioloog. Analoog aan andere disciplines als cardiologie, urologie en gynaecologie, zou de percutane echografie een integraal onderdeel van de moderne pulmonologische praktijk moeten worden.

Hoewel het logisch is dat EUS en EBUS wordt uitgevoerd in gespecialiseerde centra, vanwege hoge aanschafkosten van apparatuur en beperkte aantallen patiënten, ligt de situatie voor de percutane echografie wel degelijk anders.
Percutane echografie is goedkoop, dagelijks toepasbaar en gemakkelijk te leren. Het aanleren van percutane echografische vaardigheden, momenteel slechts beperkt tot enkele centra, zou voor iedere longarts in opleiding beschikbaar moeten zijn.
Meer diagnostiek in één hand heeft een gunstig effect op de snelheid en efficiëntie voor het stellen van de uiteindelijke diagnose.

Ofschoon diagnostische chirurgie niet gerangschikt kan worden onder de interventie pulmonologie is het wel van belang om de moderne innovaties in dit kader aan te stippen. De recente ontwikkelingen binnen de minimaal invasieve chirurgie hebben de drempel om de chirurg in te schakelen voor mediastinale en abdominale diagnostiek duidelijk verlaagd.
Video-assisted thoracoscopic surgery (VATS) was de initiële en meest belangrijke stap in dit opzicht waarmee zowel longafwijkingen als anderszins onbereikbare mediastinale en hilaire afwijkingen benaderd kunnen worden. Geleidelijk doet nu ook de robotchirurgie zijn intrede bij de VATS waardoor instrumentatie nog nauwkeuriger kan plaatsvinden en waardoor lastig bereikbare plaatsen in de thorax nog beter benaderd kunnen worden.

Pathologie en moleculaire biologie
De ontwikkelingen op het terrein van de pathologie en moleculaire biologie behoren wel tot de meest spectaculaire binnen de pulmonale oncologie. Nieuwe predictieve markers voor longkanker worden ontdekt en gevalideerd in een enorm tempo. Immuunhistochemie, fluorescent in situ hibridisatie en DNA sequencing zijn verschillende technieken die daarvoor op patiëntenmateriaal worden uitgevoerd. Meer tests brengt met zich mee dat er meer tumorcellen nodig zijn. Een ontwikkeling waarmee dit probleem ondervangen kan worden is next-generation sequencing (NGS).
Middels NGS kan op slechts zeer kleine hoeveelheden DNA en RNA analyse plaatsvinden in paraffine-ingebed materiaal. Deze methodiek is zeer waardevol als mutaties, focale amplificaties, copy number afwijkingen en sommige fusieproteïnen een predictieve waarde blijken te hebben voor de effectiviteit van een bepaalde doelgerichte (targeted) therapie. Het succes hiervan zal echter deels afhangen van de bereidheid van longartsen om grotere tumormonsters te vergaren zodat ook laagfrequente mutaties gedetecteerd kunnen worden.
Apparatuur waarmee het complete genoom gesequenced kan worden is thans commercieel verkrijgbaar waardoor alle afwijkingen in kankergenen opgespoord kunnen worden in slechts kleine hoeveelheden tumormateriaal[6].
De klinische betekenis van de meeste afwijkingen is echter nog niet bekend. Desalniettemin mag men verwachten dat de kennis van oncologische pathways snel zal toenemen met NGS, waardoor meer moleculaire doelen en resistentiemechanismen zullen worden ontdekt. Nieuwe medicamenten of combinaties van medicamenten en meer inzicht in de positieve en negatieve effecten

van medicamenten zullen de behandeling van longkanker steeds verder individualiseren.
De longarts manoeuvreert constant in een speelveld met enerzijds veranderende eisen van de patholoog ten aanzien van het aan te leveren patiëntmateriaal (afhankelijk van technische ontwikkelingen) en anderzijds een veranderend inzicht in de klinische relevantie van zekere bevindingen.

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CURRICULUM VITAE

Jos Stigt werd op 30 september 1962 geboren te Rotterdam. In 1980 deed hij eindexamen VWO aan het Strabrechtcollege te Geldrop.
Na zijn propedeuse Scheikundige Technologie gehaald te hebben aan de Technische Hogeschool te Eindhoven in 1981, werd hij ingeloot voor de studie geneeskunde. In juli 1988 werd aan de Erasmus Universiteit te Rotterdam het arts-examen behaald.
De militaire diensplicht werd vervuld als kazerne-arts te Oirschot van augustus 1988 tot oktober 1989.
De vooropleiding interne geneeskunde werd gevolgd in het Drechtsteden Ziekenhuis te Dordrecht van oktober 1989 tot 30 september 1991 waarna hij van 1 oktober 1991 tot 30 september 1995 de opleiding tot longarts volgde in het Erasmus Medisch Centrum te Rotterdam.
Sinds 1 oktober 1995 is hij werkzaam als longarts in de Isala Klinieken te Zwolle.
Jos is getrouwd met Harriette en hun dochters Isabel en Sophie werden geboren op 27 september 2001.

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